APPLICATION FOR

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SPECIFICATION

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TO ALL WHOM IT MAY CONCERN:

Be it known that:

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have invented new and useful improvements in

IL-18 BINDING PROTEINS

of which the following is a specification.

IL-18 BINDING PROTEINS

Field of the Invention

The present invention relates interleukin 18 (IL-18) binding proteins, and specifically to their uses in the prevention and/or treatment of acute and chronic inflammatory diseases.

Background of the Invention

Interleukin-18 (IL-18) was originally described in 1989 as interferon-gamma inducing factor (IGIF) and is a pro-inflammatory cytokine with various functions in addition to an ability to induce interferon gamma. These biological properties include activation of NF-κb, Fas ligand expression, the induction of both CC and CXC chemokines, and increased production of competent human immunodeficiency virus. Due to the ability of IL-18 to induce interferon gamma production in T cells and macrophages, it plays an important role in Th1-type immune responses and participates in both innate and acquired immunity. IL-18 is related to the IL-1 family in terms of both structure and function. For reviews of IL-18 structure, function and biological activity, see for example Dinarello, C. et al. (1998) *J. Leukoc. Biol.* 63:658-654; Dinarello, C.A. (1999) *Methods* 19:121-132; and Dinarello, C.A. (1999) *J. Allergy Clin. Immunol.* 103:11-24; (McInnes, I.B. et. al. (2000) *Immunology Today* 21:312-315; Nakanishi, K. et al (2001) *Ann. Rev. Immunol* 19:423-474.

Intracellular pro-IL-18 is proteolytically processed to an 18 kDa active form in endotoxin-stimulated cells by caspase 1 (Ghayur, T. et al., (1997) *Nature* 386:619-623; Gu, Y. et al., (1997) *Science* 275:206-209) and in Fas-L or bacterial DNA stimulated cells by caspases 4, 5 and 6 (Tsutsui, H. et al., (1999) *Immunity* 11:359-67; Ghayur, T., *Unpublished Observations*). Pro-IL-18 is also proteolytically processed by other proteases such as neutrophil proteinase 3 (Sugawara, S. et al., (2001) J. *Immunol.*, 167, 6568-6575), caspase 3 (Akita, K. et al., (1997) *J. Biol. Chem.* 272, 26595-26603), and serine proteases elastase and cathepsin (Gracie J. A., et al., (2003) *Journal of Leukocyte Biology* 73, 213-224). Both human and murine IL-18 lack a classical leader sequence and the mechanism of mature IL-18 release by cells is not well understood.

The biological activities of IL-18 are mediated through IL-18 binding to a heterodimeric IL-18 receptor (IL-18R) that consists of two subunits: the α-subunit (a member of the IL-1R family, also termed IL-1R-related protein-1 or IL-1Rrp1) and the β-subunit (also termed IL-18R accessory protein, IL-18AP or AcPL). The IL-18Rα subunit binds IL-18 directly, but is incapable of signal

transduction. The β-subunit does not bind IL-18 by itself, but in conjunction with the α-subunit forms the high affinity receptor (K_D = ~0.3 nM) that is required for signal transduction (Sims, J.E., (2002) *Current Opin Immunol.* 14:117-122). IL-18 signal transduction via the IL-18Rαβ complex is similar to the IL-1R and Toll like receptor (TLR) systems. IL-18R signaling uses the signal transduction molecules, such as MyD88, IRAK, TRAF6 and results in similar responses (e.g. activation of NIK, IkB kinases, NF-kB, JNK and p38 MAP kinase) as does IL-1. Requirement for IL-18Rα and signal transduction molecules in mediating IL-18 bioactivity has been confirmed using IL-18Rα subunit (Hoshino K., et al (1999) *J. Immunol.* 162:5041-5044;), MyD88 (Adachi O., et al. (1998) *Immunity* 9:143-150) or IRAK (Kanakaraj P., (1999) *J. Exp. Med.* 189:1129-1138) knockouts respectively.

Antibodies that bind IL-18 are known in the art. Mouse antibodies capable of neutralizing IL-18 are disclosed in EP 0 974 600. Human antibodies to IL-18 have been disclosed in PCT publication WO 0158956 and incorporated herein by reference. The present invention provides a novel family of binding proteins, human antibodies, and fragments thereof, capable binding IL-18, binding with high affinity, and binding and neutralizing IL-18.

Summary of the Invention

This invention pertains to IL-18 binding proteins, particularly antibodies to human IL-18, as well as methods of making and using such binding proteins. One aspect of the invention pertains to a method of regulating gene expression using a modulator of IL-18.

One aspect of this invention pertains to a binding protein comprising an antigen binding domain capable of binding human IL-18. In one embodiment the antigen binding domain comprises at least one CDR comprising an amino acid sequence selected from the group consisting of :

CDR-H1. X_1–X_2–X_3–X_4–X_5–X_6–X_7 (SEQ ID NO: 42), wherein;

X_1 is S, N, H, R, or Y;

X_2 is Y, G, R, S, or C;

X_3 is W, G, Y, D, S, V, or I;

X_4 is I, H, W, Y, M, L, or D;

X_5 is G, Y, S, N, or H;

X_6 is W, or is not present; and

X_7 is T, S, G, or is not present;

CDR-H2. X_1–X_2–X_3–X_4–X_5–X_6–X_7–X_8–X_9–X_{10}–X_{11}–X_{12}–X_{13}–X_{14}–X_{15}–X_{16}–X_{17} (SEQ ID NO: 43), wherein;

X_1 is F, Y, H, S, or V;

X_2 is I, or F;

X_3 is Y, S, or W;

X_4 is P, Y, or S;

X_5 is G, S, R, or D;

X_6 is D, or G;

X_7 is S, T, G, or R;

X_8 is E, T, I, or N;

X_9 is T, Y, N, I, K, or H;

X_{10} is R, Y, or S;

X_{11} is Y, N, or S;

X_{12} is S, P, A, or V;

X_{13} is P, S, or D;

X_{14} is T, L, or S;

X_{15} is F, K, or V;

X_{16} is Q, S, or K; and

X_{17} is G, or is not present;

CDR-H3. X_1–X_2–X_3–X_4–X_5–X_6–X_7–X_8–X_9–X_{10}–X_{11}–X_{12}–X_{13}–X_{14}–X_{15}–X_{16}–X_{17}–X_{18} (SEQ ID NO: 44), wherein;

X_1 is V, D, E, S, or C;

X_2 is G, R, D, S, K, L, Y, or A;

X_3 is S, G, Y, or R;

X_4 is G, S, Y, N, T, or D;

X_5 is W, S, A, G, Y, or T;

X_6 is Y, G, S, F, W, or N;

X_7 is P, S, F, Y, V, G, W, or V;

X_8 is Y, F, D, P, M, I, or N;

X_9 is T, W, D, L, Y, E, P, F, or G;

X_{10} is F, D, Y, H, V, Y, or is not present;

X_{11} is D, Y, F, L, or is not present;

X_{12} is I, D, Y, or is not present;

X_{13} is Y, or is not present;

X_{14} is Y, or is not present;

X_{15} is G, or is not present;

X_{16} is M, or is not present;

X_{17} is D, or is not present; and

X_{18} is V, or is not present;

CDR-L1. X_1–X_2–X_3–X_4–X_5–X_6–X_7–X_8–X_9–X_{10}–X_{11}–X_{12}–X_{13}–X_{14}–X_{15}–X_{16}–X_{17} (SEQ ID NO: 45), wherein;

X_1 is R, or K;

X_2 is A, G, or S;

X_3 is S;

X_4 is E, R, Q, or H;

X_5 is S, I, T, or N;

X_6 is I, V, L, or F;

X_7 is S, G, L, N, or R;

X_8 is S, G, Y, R, N, H, or D;

X_9 is N, G, Y, R, or S;

X_{10} is L, Y, S, or D;

X_{11} is A, L, N, V, G, or D;

X_{12} is A, N, E, K, G, or is not present;

X_{13} is K, T, N, or is not present;

X_{14} is N, Y, T, or is not present;

X_{15} is Y, L, or is not present;

X_{16} is L, C, Y, or is not present; and

X_{17} is A, D, or is not present;

CDR-L2. X_1–X_2–X_3–X_4–X_5–X_6–X_7 (SEQ ID NO: 46), wherein;

X_1 is T, G, S, W, or E;

X_2 is A, V, T, I, or L;

X_3 is S, or F;

X_4 is T, I, N, S, R, or Y;

X_5 is R, or L;

X_6 is A, Q, E, or F; and

X_7 is T, or S;

and

CDR-L3. X_1–X_2–X_3–X_4–X_5–X_6–X_7–X_8–X_9–X_{10} (SEQ ID NO: 47), wherein;

X_1 is Q, or M;

X_2 is Q, H, or Y;

X_3 is Y, N, G, S, or R;

X_4 is N, H, Y, D, G, V, L, or I;

X_5 is N, G, I, Y, S, Q, F, or E;

X_6 is W, S, T, L, I, or F;

X_7 is P, L, T, D, or I;

X_8 is S, L, P, C, W, I, or F;

X_9 is I, T, S, or is not present; and

X_{10} is T, or is not present.

Preferably, the antigen binding domain comprises at least one CDR comprising an amino acid sequence selected from the group consisting of Residues 31-35 of SEQ ID NO.:6; Residues 50-66 of SEQ ID NO.:6; Residues 99-110 of SEQ ID NO.:6; Residues 24-34 of SEQ ID NO.:7; Residues 50-56 of SEQ ID NO.:7; Residues 89-98 of SEQ ID NO.:7; Residues 31-37 of SEQ ID NO.:8; Residues 52-67 of SEQ ID NO.:8; Residues 100-110 of SEQ ID NO.:8; Residues 24-35 of SEQ ID NO.:9; Residues 21-27 of SEQ ID NO.:9; Residues 90- 98 of SEQ ID NO.:9; Residues 31-35 of SEQ ID NO.:10; Residues 50-65 of SEQ ID NO.:10; Residues 98-107 of SEQ ID NO.:10; Residues 24-34 of SEQ ID NO.:11; Residues 50-56 of SEQ ID NO.:11; Residues 89-97 of SEQ ID NO.:11; Residues 31-37 of SEQ ID NO.:12; Residues 52-67 of SEQ ID NO.:12; Residues 100-108 of SEQ ID NO.:12; Residues 24-35 of SEQ ID NO.:13; Residues 51-57 of SEQ ID NO.:13; Residues 90-98 of SEQ ID NO.:13; Residues 31-35 of SEQ ID NO.:14; Residues 50-66 of SEQ ID NO.:14; Residues 99-111 of SEQ ID NO.:14; Residues 24-40 of SEQ ID NO.:15; Residues 56-62 of SEQ ID NO.:15; Residues 95-103 of SEQ ID NO.:15; Residues 31-37 of SEQ ID NO.:16;.Residues 52-67 of SEQ ID NO.:16; Residues 100-109 of SEQ ID NO.:16; Residues 24-35 of SEQ ID NO.:17; Residues 51-57 of SEQ ID NO.:17; Residues 90-98 of SEQ ID NO.:17; Residues 31-35 of SEQ ID NO.:18; Residues 20-36 of SEQ ID NO.:18; Residues 99-108 of SEQ ID NO.:18; Residues 24-34 of SEQ ID NO.:19; Residues 50-56 of SEQ ID NO.:19; Residues 89-97 of SEQ ID NO.:19; Residues 31-35 of SEQ ID NO.:20; Residues 52-67 of SEQ ID NO.:20; Residues 100-108 of SEQ ID NO.:20; Residues 24-35 of SEQ ID NO.:21; Residues 51-57 of SEQ ID NO.:21; Residues 90-98 of SEQ ID NO.:21; Residues 31-35 of SEQ ID NO.:22; Residues 50-66 of SEQ ID NO.:22; Residues 99-116 of SEQ ID NO.:22; Residues 24-39 of SEQ ID NO.:23;

Residues 55-61 of SEQ ID NO.:23; Residues 94-102 of SEQ ID NO.:23; Residues 31-37 of SEQ ID NO.:24; Residues 52-67 of SEQ ID NO.:24; Residues 100-109 of SEQ ID NO.:24; Residues 24-35 of SEQ ID NO.:25; Residues 51-57 of SEQ ID NO.:25; Residues 90-98 of SEQ ID NO.:25; Residues 31-37 of SEQ ID NO.:26; Residues 52-67 of SEQ ID NO.:26; Residues 100-109 of SEQ ID NO.:26; Residues 24-35 of SEQ ID NO.:27; Residues 51-57 of SEQ ID NO.:27; Residues 90-98 of SEQ ID NO.:27; Residues 31-37 of SEQ ID NO.:28; Residues 52-67 of SEQ ID NO.:28; Residues 100-108 of SEQ ID NO.:28; Residues 24-35 of SEQ ID NO.:29; Residues 51-57 of SEQ ID NO.:29; Residues 90-98 of SEQ ID NO.:29; Residues 31-37 of SEQ ID NO.:30; Residues 52-67 of SEQ ID NO.:30; Residues 99-109 of SEQ ID NO.:30; Residues 24-35 of SEQ ID NO.:31; Residues 51-57 of SEQ ID NO.:31; Residues 90-98 of SEQ ID NO.:31; Residues 31-37 of SEQ ID NO.:32; Residues 52-67 of SEQ ID NO.:32; Residues 100-109 of SEQ ID NO.:32; Residues 24-35 of SEQ ID NO.:33; Residues 51-57 of SEQ ID NO.:33; Residues 90-98 of SEQ ID NO.:33; Residues 31-37 of SEQ ID NO.:34; Residues 52-67 of SEQ ID NO.:34; Residues 100-108 of SEQ ID NO.:34; Residues 24-35 of SEQ ID NO.:35; Residues 51-57 of SEQ ID NO.:35; Residues 90-98 of SEQ ID NO.:35; Residues 31-35 of SEQ ID NO.:36; Residues 50-66 of SEQ ID NO.:36; Residues 99-116 of SEQ ID NO.:36; Residues 24-39 of SEQ ID NO.:37; Residues 55-61 of SEQ ID NO.:37; Residues 94-102 of SEQ ID NO.:37; Residues 31-35 of SEQ ID NO.:38; Residues 50-66 of SEQ ID NO.:38; Residues 99-108 of SEQ ID NO.:38; Residues 24-35 of SEQ ID NO.:39; Residues 51-57 of SEQ ID NO.:39; Residues 90-98 of SEQ ID NO.:39; Residues 31-37 of SEQ ID NO.:40; Residues 52-67 of SEQ ID NO.:40; Residues 97-109 of SEQ ID NO.:40; Residues 24-40 of SEQ ID NO.:41; Residues 56-62 of SEQ ID NO.:41; Residues 95-103 of SEQ ID NO.:41. Preferably the binding protein comprises at least 3 CDRs.

In another preferred embodiment the binding protein comprises a V_H domain. Preferably the V_H domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6; SEQ ID NO: 8; SEQ ID NO: 10; SEQ ID NO: 12; SEQ ID NO: 14; SEQ ID NO: 16; SEQ ID NO: 18; SEQ ID NO: 20; SEQ ID NO: 22; SEQ ID NO: 24; SEQ ID NO: 26; SEQ ID NO: 28; SEQ ID NO: 30; SEQ ID NO: 32; SEQ ID NO: 34; SEQ ID NO: 36; SEQ ID NO: 38; and SEQ ID NO: 40. In another embodiment the binding protein comprises a V_L domain. Preferably the V_L domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 7; SEQ ID NO: 9; SEQ ID NO: 11; SEQ ID NO: 13; SEQ ID NO: 15; SEQ ID NO: 17; SEQ ID NO: 19; SEQ ID NO: 21; SEQ ID NO: 23; SEQ ID NO: 25; SEQ ID NO: 27; SEQ ID NO: 29; SEQ ID NO: 31; SEQ ID NO: 33; SEQ ID NO: 35; SEQ ID NO: 37; SEQ ID NO: 39; and SEQ ID NO: 41.

In a preferred embodiment the binding protein comprises a V_H and a V_L domain. More preferably the binding protein comprises a V_H domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 6; SEQ ID NO: 8; SEQ ID NO: 10; SEQ ID NO: 12; SEQ ID NO: 14; SEQ ID NO: 16; SEQ ID NO: 18; SEQ ID NO: 20; SEQ ID NO: 22; SEQ ID NO: 24; SEQ ID NO: 26; SEQ ID NO: 28; SEQ ID NO: 30; SEQ ID NO: 32; SEQ ID NO: 34; SEQ ID NO: 36; SEQ ID NO: 38; and SEQ ID NO: 40 and a V_L domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 7; SEQ ID NO: 9; SEQ ID NO: 11; SEQ ID NO: 13; SEQ ID NO: 15; SEQ ID NO: 17; SEQ ID NO: 19; SEQ ID NO: 21; SEQ ID NO: 23; SEQ ID NO: 25; SEQ ID NO: 27; SEQ ID NO: 29; SEQ ID NO: 31; SEQ ID NO: 33; SEQ ID NO: 35; SEQ ID NO: 37; SEQ ID NO: 39; and SEQ ID NO: 41. Most preferably the binding protein comprises a V_L domain comprising an amino acid sequence of SEQ ID NO: 7, and a V_H domain comprising an amino acid sequence of SEQ ID NO: 6.

In another embodiment the binding protein further comprises a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgM constant domain; a human IgG1 constant domain; a human IgG2 constant domain; a human IgG3 constant domain; a human IgG4 constant domain; a human IgE constant domain and a human IgA constant domain. Preferably the heavy chain immunoglobulin constant region domain is a human IgG1 constant domain. Preferably at least one amino acid residue is replaced in the heavy chain constant region domain such that effector functions of the antibody are altered. More preferably the human IgG1 constant domain comprises amino acid sequence selected from the group consisting of SEQ ID NO.:2, and SEQ ID NO.:3.

In another embodiment the binding protein further comprises a light chain immunoglobulin constant domain selected from the group consisting of a human Ig kappa constant domain; and a human Ig lambda constant domain. Preferably the human Ig kappa constant domain comprises amino acid sequence SEQ ID NO.:4 and the human Ig lambda constant domain comprises amino acid sequence SEQ ID NO.:5.

In another embodiment the binding protein comprises an Ig constant heavy region having an amino acid sequence selected from the group consisting of: SEQ ID NO:2, and SEQ ID NO: 3; an IG constant light region having an amino acid sequence selected from the group consisting of: SEQ ID NO:4, and SEQ ID NO: 5; an Ig variable heavy region having an amino acid sequence of SEQ ID NO:6; and an Ig variable light region having an amino acid sequence of SEQ ID NO:7

In another embodiment the binding protein comprises an Ig constant heavy region having an amino acid sequence of SEQ ID NO: 3; an IG constant light region having an amino acid sequence of

SEQ ID NO:4; an Ig variable heavy region having an amino acid sequence of SEQ ID NO:6; and an Ig variable light region having an amino acid sequence of SEQ ID NO:7.

In another embodiment the binding is selected from the group consisting of an immunoglobulin molecule or functional variants thereof known in the art, which variants retain the characteristic binding property of the binding protein. Examples of specific immunoglobulin embodiments include but are not limited to an scFv; a monoclonal antibody; a human antibody; a chimeric antibody; a humanized antibody; a single domain antibody; a Fab fragment; an Fab' fragment; an F(ab')2; an Fv; a disulfide linked Fv, and a bispecific or dual specific antibody. Most preferably the binding protein is a human antibody.

Another aspect of the invention provides a neutralizing binding protein comprising any one of the binding proteins disclosed above wherein the neutralizing binding protein is capable of neutralizing IL-18. Preferably the neutralizing binding protein is capable of neutralizing any one of pro-human IL-18; mature-human IL-18 or truncated-human IL-18. In another embodiment the neutralizing binding protein diminishes the ability of IL-18 to bind to its receptor. Preferably the neutralizing binding protein diminishes the ability of pro-human IL-18; mature-human IL-18 or truncated-human IL-18 to bind to its receptor.

In another embodiment the neutralizing binding protein is capable of inhibiting one or more of IL-18 biological activities selected from the group consisting of, Th1 modulation; Th2 modulation (Nakanishi K., et al (2001) Cytokine and Growth Factor Rev. 12:53-72); Nk modulation; neutrophil modulation; monocyte-macrophage lineage modulation; neutrophil modulation; eosinophil modulation; B-cells modulation; cytokine modulation; chemokine modulation; adhesion molecule modulation; and cell recruitment modulation.

In a preferred embodiment the neutralizing binding protein has a dissociation constant (K_D) selected from the group consisting of: at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; and at most 10^{-13} M.

In another embodiment the neutralizing binding protein has an on rate selected from the group consisting of: at least about $10^2 M^{-1}s^{-1}$; at least about $10^3 M^{-1}s^{-1}$; at least about $10^4 M^{-1}s^{-1}$; at least about $10^5 M^{-1}s^{-1}$; and at least about $10^6 M^{-1}s^{-1}$.

In yet another embodiment the neutralizing binding protein has an off rate selected from the group consisting of: at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; and at most about $10^{-6}s^{-1}$.

Another aspect of the invention provides a labeled binding protein comprising any one of the binding proteins disclosed above wherein the binding protein is conjugated to a detectable label.

Preferably the detectable label is selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label and biotin. More preferably the radiolabel is ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm.

Another aspect of the invention provides a conjugate protein comprising any one of the binding proteins disclosed above wherein said binding protein is conjugated to a therapeutic or cytotoxic agent. Preferably the therapeutic or cytotoxic agent is selected from the group consisting of an anti-metabolite; an alkylating agent; an antibiotic; a growth factor; a cytokine; an anti-angiogenic agent; an anti-mitotic agent; an anthracycline; toxin; and an apoptotic agent.

One embodiment pertains to an isolated nucleic acid encoding any one of the binding proteins disclosed above. A further embodiment provides a vector comprising the isolated nucleic acid disclosed above wherein said vector is selected from the group consisting of pcDNA; pTT (Durocher et al., *Nucleic Acids Research* 2002, Vol 30, No.2); pTT3 (pTT with additional multiple cloning site; pEFBOS (Mizushima, S. and Nagata, S., (1990) *Nucleic acids Research* Vol 18, No. 17); pBV; pJV; and pBJ.

In another embodiment a host cell is transformed with the vector. Preferably the host cell is a prokaryotic cell. More preferably the host cell is E.Coli. In a related embodiment the host cell is an eukaryotic cell. Preferably the eukaryotic cell is selected from the group consisting of protist cell, animal cell, plant cell and fungal cell. More preferably the host cell is a mammalian cell including, but not limited to, CHO and COS; or a fungal cell such as Saccharomyces cerevisiae; or an insect cell such as Sf9.

Another aspect of the invention provides a method of producing a binding protein that binds human IL-18, comprising culturing any one of the host cells disclosed above in a culture medium under conditions sufficient to produce a binding protein that binds human IL-18. Another embodiment provides a binding protein produced according to the method disclosed above.

Another aspect of the invention provides a crystallized binding protein comprising any one of the binding proteins disclosed above, wherein the binding protein exists as a crystal. Preferably the crystal is a carrier- free pharmaceutical controlled release crystal. In one embodiment the binding protein that exists as a crystal has a greater half-life in vivo than the soluble counterpart of the binding protein. In another embodiment the binding protein retains its biological activity after crystallization.

One embodiment provides a composition for the release of a binding protein wherein the composition comprises a formulation which in turn comprises a crystallized binding protein as disclosed above and an ingredient; and at least one polymeric carrier. Preferably the polymeric

carrier is a polymer selected from one or more of the group consisting of: poly (acrylic acid), poly (cyanoacrylates), poly (amino acids), poly (anhydrides), poly (depsipeptide), poly (esters), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (b-hydroxybutryate), poly (caprolactone), poly (dioxanone); poly (ethylene glycol), poly ((hydroxypropyl) methacrylamide, poly [(organo)phosphazene], poly (ortho esters), poly (vinyl alcohol), poly (vinylpyrrolidone), maleic anhydride- alkyl vinyl ether copolymers, pluronic polyols, albumin, alginate, cellulose and cellulose derivatives, collagen, fibrin, gelatin, hyaluronic acid, oligosaccharides, glycaminoglycans, sulfated polyeaccharides, blends and copolymers thereof. Preferably the ingredient is selected from the group consisting of albumin, sucrose, trehalose, lactitol, gelatin, hydroxypropyl-β- cyclodextrin, methoxypolyethylene glycol and polyethylene glycol. Another embodiment provides a method for treating a mammal comprising the step of administering to the mammal an effective amount of the composition disclosed above.

Another aspect of the invention provides a method for regulating gene expression of a gene of interest comprising the steps of providing an IL-18 polypeptide or an IL-18 modulator; and contacting the polypeptide or modulator to a cell wherein the gene of interest is selected from the group consisting of genes identified by Genbank Identification numbers:

NM_000389, NM_002198, NM_002163, NM_006144, NM_006515, NM_007185, NM_002288,
NM_003661, NM_021958, NM_001335, Hs.382006, NM_020125, NM_007210, NM_021798,
NM_013324, M11313, D88152, NM_001103, U37519, NM_000697, J03600,
NM_014578, S66793, U47054, L19871, M81181, NM_001188, U15460,
NM_014417, Z23115, NM_001713, U45878, U37546, U72649, U49187,
J03507, U50360 XM_071866, NM_005623, Z32765, Z11697, XM_071866,
U51096, M83667, D87469, L07765, U66468, X14830, L29217,
X15880, NM_001851, M27691, M37435, X13589, X16866, X59131,
NM_004393, U73328, L19267, U53445, X68277, U48807, NM_001950,
U87269, M57730, X52541, J04076, X63741, L07077, M62831,
M60830, U53786, NM_001988, NM_000141, M23668, U60062, NM_000141,
U49973, U89995, U27326, A28102, M25667, L34357, U19523,
L01406, U03486, X68285, Z18859, D49958, D43772, AC000099,
M57731, X53800, M91036, D16583, X64877, X58431, M16937,
NM_014468, X92814, L19314, M26665, D10995, L41147, M24283,
S81914, J03171, J00219, NM_000619, NM_000585, U31628, X04500,
M27492, X01057, M26062, Y00081, Y00787, Z31695, X06256,
X57206, U20734, NM_014879, D31762, D42038, NM_005551, NM_014846,
X06182, NM_005551, X07730, M13955, M57710, S83362, NM_002314,
NM_005569, U49957, U89922, X14008, U59914, D14497, X59727,
NM_000429, U43944, X72755, NM_021230, NM_005951, X78710, X70991,
M32011, S77763, M58603, S76638, M69043, U91616, D86425,
L13740, U44848, U79251, M27288, AF000234, D50640, L20971,
L10343, U77735, NM_003579, U17034, AB000584, X63131, D11428,
NM_032940, NM_005035, NM_003579, M18255, L01087, D38128, Y10375,

D15049, M31166, U59877, NM_003579, U64675, S57153, NM_002903, NG_000013, X75042, M83221, NM_000537, U22314, S59049, U70426, U22377, U38480, L10338, M23178, M69203, NM_005409, D79206, NM_005065, NM_004186, J03764, NM_006802, D89077, NM_003037, M91463, D82326, L05568, U96094, X83301, D21267, L31529, M62800, NM_021014, Z35093, NM_005816, L25444, M95787, NM_005421, L47345, M57732, NM_003205, M96956, U19878, M92357, M59465, X83490, U37518, NM_003294, U19261, U78798, S69790, U53476, L15309, U78722, X57809, U79249, AB000464, X77744, U79248, AI420129, HG2981-HT3127, HG3548-HT3749, HG870-HT870, HG4333-HT4603, HG3111-HT3287, HG4593-HT4998, HG961-HT961, HG1877-HT1917, HG3115-HT3291, HG4115-HT4385, and HG3925-HT4195.

Preferably the modulator is an antagonist. More preferably the modulator is a binding protein or a neutralizing binding protein.

The invention also provides a pharmaceutical composition comprising a binding protein or a neutralizing binding protein as disclosed above and a pharmaceutically acceptable carrier. In a further embodiment the pharmaceutical composition comprises at least one additional therapeutic agent for treating a disorder in which IL-18 activity is detrimental. Preferably the additional agent is selected from the group consisting of: angiogenesis inhibitors (including but not limited to anti-VEGF antibodies or VEGF-trap); kinase inhibitors (including but not limited to KDR and TIE-2 inhibitors); co-stimulation molecule blockers (including but not limited to anti-B7.1, anti-B7.2, CTLA4-Ig, anti-CD20); adhesion molecule blockers (including but not limited to anti-LFA-1 Abs, anti-E/L selectin Abs, small molecule inhibitors); anti-cytokine antibody or functional fragment thereof (including but not limited to anti-IL-12, anti-TNF, anti-IL-6/cytokine receptor antibodies); methotrexate; corticosteroids; cyclosporin; rapamycin; FK506; and non-steroidal anti-inflammatory agents.

In another aspect, the invention provides a method for inhibiting human IL-18 activity comprising contacting human IL-18 with a binding protein disclosed above such that human IL-18 activity is inhibited. In a related aspect the invention provides a method for inhibiting human IL-18 activity in a human subject suffering from a disorder in which IL-18 activity is detrimental, comprising administering to the human subject a binding protein disclosed above such that human IL-18 activity in the human subject is inhibited and treatment is achieved. Preferably the disorder is selected from the group comprising rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, and septic arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis

scleroderma, graft versus host disease, organ transplant rejection (including but not limited to bone marrow and solid organ rejection), acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthopathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthopathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis,

primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasulitis of the kidneys, Lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjögren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis ,vitiligo, acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatatis, idiosyncratic liver disease, Drug-Induced hepatitis, Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (*e.g.*, depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma).

In another aspect the invention provides a method of treating a patient suffering from a disorder in which IL-18 is detrimental comprising the step of administering any one of the binding proteins disclosed above before, concurrent, or after the administration of a second agent, as discussed above.

Detailed Description of the Invention

This invention pertains to IL-18 binding proteins, particularly anti-Il-18 antibodies, or antigen-binding portions thereof, that bind thereto. Various aspects of the invention relate to antibodies and antibody fragments, and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such antibodies and fragments. Methods of using the antibodies of the invention to detect human IL-18, to inhibit human IL-18 activity, either in vitro or in vivo, and to regulate gene expression are also encompassed by the invention. This invention also pertains to a truncated IL-18. In related aspects the invention also pertains to nucleic acids, recombinant expression vectors and host cells for making truncated IL-18.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. In this application, the use of "or" means "and/or" unless

stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

That the present invention may be more readily understood, select terms are defined below.

The term "Polypeptide" as used herein, refers to any polymeric chain of amino acids. The terms "peptide" and "protein" are used interchangeably with the term polypeptide and also refer to a polymeric chain of amino acids. The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide that by virtue of its origin or source of derivation is not associated with naturally associated components that accompany it in its native state; is substantially free of other proteins from the same species; is expressed by a cell from a different species; or does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

The term "recovering" as used herein, refers to the process of rendering a chemical species such as a polypeptide substantially free of naturally associated components by isolation, e.g., using protein purification techniques well known in the art.

The term "IL-18" as used herein, refers to the cytokine also known as interferon-gamma inducing factor (IGIF), that is a pro-inflammatory cytokine, that exhibits various functions in addition to an ability to induce interferon gamma. The term "human IL-18" used interchangeably with the term "hIL-18" encompasses polypeptide of SEQ ID NO: 1 and fragments thereof, including but not limited to, pro-human IL-18, mature human IL-18, and any truncated human IL-18 that retains a biological activity of IL-18 as described herein. The term "pro-human IL-18" as used herein, refers to a polypeptide of SEQ ID NO: 1. The term "mature human IL-18" as used herein, refers to residues 37-193 of SEQ ID NO: 1, and the term "truncated human IL-18 as used herein, refers to residues 59-193 of SEQ ID NO: 1. Preferably the IL-18, and fragments thereof, are biologically active. The term "recombinant human IL-18" or "rhIL-18" as used herein, refers to human IL-18 generated *in vitro* using recombinant DNA techniques.

"Biological activity of IL-18" as used herein, refers to all inherent biological properties of the cytokine IL-18. Biological properties of IL-18 include but are not limited to binding IL-18 receptor; promoting maturation and activation of Th1 and Tc1 cells; promoting production of cytokines such as TNF, IFNγ and IL-1β by several cell types; promoting macrophages to release cytokines such as TNF and IFNγ, produce NO; promoting FasL expression, cytotoxicity and cytokine release (IFNγ) from NK cells; promoting cytokine/chemokine release, respiratory burst, granule release, adhesion molecule expression in Neutrophils; promoting endothelial cells to migrate and thereby promote angiogenesis; promoting GAG release, MMP and NO production in Chondrocytes; promoting COX2 expression in some cells; and reducing cell proliferation in some cells.

The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

The term "antibody", as used herein, broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative anitbody formats are known in the art. Nonlimiting embodiments of which are discussed below.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, hIL-18). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883) . Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Such

antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M., *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S.M., *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds hIL-18 is substantially free of antibodies that specifically bind antigens other than hIL-18). An isolated antibody that specifically binds hIL-18 may, however, have cross-reactivity to other antigens, such as IL-18 molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further in Section II C, below), antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom H.R., (1997) *TIB Tech.* 15:62-70; Azzazy H., and Highsmith W.E., (2002) *Clin. Biochem.* 35:425-445; Gavilondo J.V., and Larrick J.W. (2002) *BioTechniques* 29:128-145;

Hoogenboom H., and Chames P. (2000) *Immunology Today* 21:371-378), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann S-A., and Green L.L. (2002) *Current Opinion in Biotechnology* 13:593-597; Little M. et al (2000) *Immunology Today* 21:364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

The term "chimeric antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

The term "CDR-grafted antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of V_H and/or VL are replaced with CDR sequences of another species, such as antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs (*e.g.*, CDR3) has been replaced with human CDR sequences.

The term "humanized antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a non-human species (*e.g.*, a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", *i.e.*, more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences.

As used herein, the term "hIL-18 neutralizing binding protein" refers to a protein that specifically binds hIL-18 and neutralizes a biological activity of hIL-18. Preferably a neutralizing binding protein is a neutralizing antibody whose binding to hIL-18 results in inhibition of a biological activity of hIL-18. Preferably the neutralizing binding protein binds hIL-18 and reduces a biologically activity of IL-18 by at least about 20%, 40%, 60%, 80%, 85% or more. This inhibition of a biological activity of hIL-18 by a neutralizing binding protein can be assessed by measuring one

or more indicators of hIL-18 biological activity. These indicators of hIL-18 biological activity can be assessed by one or more of several standard *in vitro* or *in vivo* assays known in the art.

The term "epitope" includes any polypeptide determinant capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules.

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jönsson, U., *et al.* (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., *et al.* (1991) *Biotechniques* 11:620-627; Johnsson, B., *et al.* (1995) *J. Mol. Recognit.* 8:125-131; and Johnnson, B., *et al.* (1991) *Anal. Biochem.* 198:268-277.

The term "K_{on}", as used herein, is intended to refer to the on rate constant for association of an antibody to the antigen to form the antibody/antigen complex as is known in the art.

The term "K_{off}", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex as is known in the art.

The term "K_d", as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction as is known in the art.

The term "labeled binding protein" as used herein, refers to a protein with a label incorporatedthat provides for the identification of the binding protein. Preferably, the label is a detectable marker, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm); fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for

secondary antibodies, metal binding domains, epitope tags); and magnetic agents, such as gadolinium chelates.

The term "conjugate binding protein" refers to a binding protein chemically linked to a second chemical moiety, such as a therapeutic or cytotoxic agent. The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. Preferably the therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

The term "crystallized binding protein" as used herein, refers to a polypeptide that exists in the form of a crystal. Crystals are one form of the solid state of matter, which is distinct from other forms such as the amorphous solid state or the liquid crystalline state. Crystals are composed of regular, repeating, three-dimensional arrays of atoms, ions, molecules (e.g., proteins such as antibodies), or molecular assemblies (e.g., antigen/antibody complexes). These three-dimensional arrays are arranged according to specific mathematical relationships that are well-understood in the field. The fundamental unit, or building block, that is repeated in a crystal is called the asymmetric unit. Repetition of the asymmetric unit in an arrangement that conforms to a given, well-defined crystallographic symmetry provides the "unit cell" of the crystal. Repetition of the unit cell by regular translations in all three dimensions provides the crystal. See Giege, R. and Ducruix, A. Barrett, Crystallization of Nucleic Acids and Proteins, a Practical Approach, 2nd ea., pp. 20 1-16, Oxford University Press, New York, New York, (1999)."

The term "polynucleotide" as referred to herein means a polymeric form of two or more nucleotides, either ribonucleotides or deoxvnucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA but preferably is double-stranded DNA.

The term "isolated polynucleotide" as used herein shall mean a polynucleotide (e.g., of genomic, cDNA, or synthetic origin, or some combination thereof) that, by virtue of its origin , the "isolated polynucleotide": is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature; is operably linked to a polynucleotide that it is not linked to in nature; or does not occur in nature as part of a larger sequence.

The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. "Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. The term "expression control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include components whose presence is essential for expression and

processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

"Transformation", as defined herein, refers to any process by which exogenous DNA enters a host cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which exogenous DNA has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell, but, to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Preferably host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life. Preferred eukaryotic cells include protist, fungal, plant and animal cells. Most preferably host cells include but are not limited to the prokaryotic cell line E.Coli; mammalian cell lines CHO and COS; the insect cell line Sf9; and the fungal cell Saccharomyces cerevisiae.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose.

"Transgenic organism", as known in the art and as used herein, refers to an organism having cells that contain a transgene, wherein the transgene introduced into the organism (or an ancestor of the organism) expresses a polypeptide not naturally expressed in the organism. A "transgene" is a

DNA construct, which is stably and operably integrated into the genome of a cell from which a transgenic organism develops, directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic organism.

The term "regulate"and "modulate" are used interchangeably, and, as used herein, refers to a change or an alteration in the activity of a molecule of interest (e.g., the biological activity of hIL-18). Modulation may be an increase or a decrease in the magnitude of a certain activity or function of the molecule of interest. Exemplary activities and functions of a molecule include, but are not limited to, binding characteristics, enzymatic activity, cell receptor activation, and signal transduction.

Correspondingly, the term "modulator," as used herein, is a compound capable of changing or altering an activity or function of a molecule of interest (e.g., the biological activity of hIL-18). For example, a modulator may cause an increase or decrease in the magnitude of a certain activity or function of a molecule compared to the magnitude of the activity or function observed in the absence of the modulator. In certain embodiments, a modulator is an inhibitor, which decreases the magnitude of at least one activity or function of a molecule. Exemplary inhibitors include, but are not limited to, proteins, peptides, antibodies, peptibodies, carbohydrates or small organic molecules. Peptibodies are described, e.g., in WO01/83525.

The term "agonist", as used herein, refers to a modulator that, when contacted with a molecule of interest, causes an increase in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the agonist. Particular agonists of interest may include, but are not limited to, IL-18 polypeptides or polypeptides, nucleic acids, carbohydrates, or any other molecules that bind to hIL-18.

The term "antagonist" or "inhibitor", as used herein, refer to a modulator that, when contacted with a molecule of interest causes a decrease in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the antagonist. Particular antagonist of interest include those that block or modulate the biological or immunological activity of hIL-18. Antagonists and inhibitors of hIL-18 may include, but are not limited to, proteins, nucleic acids, carbohydrates, or any other molecules which bind to hIL-18.

The term "sample", as used herein, is used in its broadest sense. A "biological sample", as used herein, includes, but is not limited to, any quantity of a substance from a living thing or formerly living thing. Such living things include, but are not limited to, humans, mice, rats,

monkeys, dogs, rabbits and other animals. Such substances include, but are not limited to, blood, serum, urine, synovial fluid, cells, organs, tissues, bone marrow, lymph nodes and spleen.

I. Human Antibodies that Bind Human IL-18.

One aspect of the present invention provides isolated human antibodies, or antigen-binding portions thereof, that bind to IL-18with high affinity, a low off rate and high neutralizing capacity. Preferably, the antibodies, or portions thereof, are isolated antibodies. Preferably, the human antibodies of the invention are neutralizing human anti-IL-18 antibodies.

A. Method of making anti IL-18 antibodies

Antibodies of the present invention may be made by any of a number of techniques known in the art. A particularly preferred method for generating anti-IL-18 antibodies of the invention include using XENOMOUSE transgenic mice, and using hybridoma and SLAM cellular manipulation techniques (Abgenix, Inc., Fremont, CA) known in the art for preparing antibodies, and using antigens comprising the IL-18 peptide described in Example 3.2, i.e., human IL-18 comprising amino acid sequence of SEQ ID NO. 1 and fragments thereof.

In one embodiment of the instant invention, human antibodies are produced by immunizing a non-human animal comprising some, or all, of the human immunoglobulin locus with an IL-18 antigen. In a preferred embodiment, the non-human animal is a XENOMOUSE transgenic mouse, an engineered mouse strain that comprises large fragments of the human immunoglobulin loci and is deficient in mouse antibody production. *See, e.g.,* Green et al. *Nature Genetics* 7:13-21 (1994) and United States Patents 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598 and 6,130,364. See also WO 91/10741, published July 25,1991, WO 94/02602, published February 3, 1994, WO 96/34096 and WO 96/33735, both published October 31, 1996, WO 98/16654, published April 23, 1998, WO 98/24893, published June 11, 1998, WO 98/50433, published November 12, 1998, WO 99/45031, published September 10, 1999, WO 99/53049, published October 21, 1999, WO 00 09560, published February 24, 2000 and WO 00/037504, published June 29, 2000. The XENOMOUSE transgenic mouse produces an adult-like human repertoire of fully human antibodies, and generates antigen-specific human Mabs. The XENOMOUSE transgenic mouse contains approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and x light chain loci. See Mendez et al., *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J. Exp. Med.* 188:483-495 (1998), the disclosures of which are hereby incorporated by reference.

The invention also provides a method for making anti-IL-18 antibodies from non-human, non-mouse animals by immunizing non-human transgenic animals that comprise human immunoglobulin loci. One may produce such animals using the methods described immediately above. The methods disclosed in these patents may be modified as described in United States Patent 5,994,619. In a preferred embodiment, the non-human animals may be rats, sheep, pigs, goats, cattle or horses.

In another embodiment, the non-human animal comprising human immunoglobulin gene loci are animals that have a "minilocus" of human immunoglobulins. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of individual genes from the Ig locus. Thus, one or more V_H genes, one or more DH genes, one or more JH genes, a mu constant region, and a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described, *inter alia,* in U.S. Patent No. 5,545,807, 5,545,806, 5,625,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, 5,814,318, 5,591,669, 5,612,205, 5,721,367, 5,789,215, and 5,643,763, hereby incorporated by reference.

An advantage of the minilocus approach is the rapidity with which constructs including portions of the Ig locus can be generated and introduced into animals. However, a potential disadvantage of the minilocus approach is that there may not be sufficient immunoglobulin diversity to support full B-cell development, such that there may be lower antibody production.

In order to produce a human anti-IL-18 antibody, a non-human animal comprising some or all of the human immunoglobulin loci is immunized with an IL-18 antigen and the antibody or the antibody-producing cell is isolated from the animal. The IL-18 antigen may be isolated and/or purified IL-18 and is preferably a human IL-18. In another embodiment, the IL-18 antigen is a fragment of IL-18, preferably mature IL-18. In another embodiment, the IL-18 antigen is a fragment that comprises at least one epitope of IL-18.

Immunization of animals may be done by any method known in the art. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, New York: Cold Spring Harbor Press, 1990. Methods for immunizing non-human animals such as mice, rats, sheep, goats, pigs, cattle and horses are well known in the art. See, e.g., Harlow and Lane and United States Patent 5,994,619. In a preferred embodiment, the IL-18 antigen is administered with a adjuvant to stimulate the immune response. Such adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune

system. Preferably, if a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks.

Example 2.2.A provides a protocol for immunizing a XENOMOUSE transgenic mouse with human IL-18 in phosphate-buffered saline.

B. Production of Antibodies and Antibody-Producing Cell Lines

After immunization of an animal with an IL-18 antigen, antibodies and/or antibody-producing cells may be obtained from the animal. An anti- IL-18 antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the anti-IL-18 antibodies may be purified from the serum. Serum or immunoglobulins obtained in this manner are polyclonal, thus having a heterogeneous array of properties.

In another embodiment, antibody-producing immortalized hybridomas may be prepared from the immunized animal. After immunization, the animal is sacrificed and the splenic B cells are fused to immortalized myeloma cells as is well known in the art. See, e.g., Harlow and Lane, *supra.* In a preferred embodiment, the myeloma cells do not secrete immunoglobulin polypeptides (a non-secretory cell line). After fusion and antibiotic selection, the hybridomas are screened using IL-18, or a portion thereof, or a cell expressing IL-18. In a preferred embodiment, the initial screening is performed using an enzyme-linked immunoassay (ELISA) or a radioimmunoassay (RIA), preferably an ELISA. An example of ELISA screening is provided in WO 00/37504, herein incorporated by reference.

Anti- IL-18 antibody-producing hybridomas are selected, cloned and further screened for desirable characteristics, including robust hybridoma growth, high antibody production and desirable antibody characteristics, as discussed further below. Hybridomas may be cultured and expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro.* Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

Preferably, the immunized animal is a non-human animal that expresses human immunoglobulin genes and the splenic B cells are fused to a myeloma derived from the same species as the non-human animal. More preferably, the immunized animal is a XENOMOUSE transgenic mouse and the myeloma cell line is a non-secretory mouse myeloma, such as the myeloma cell line is P3X63Ag8.653 (see, e.g., Example 2.2.B).

In one aspect, the invention provides hybridomas that produce human anti- IL-18 antibodies. In a preferred embodiment, the hybridomas are mouse hybridomas, as described above. In another preferred embodiment, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas, in which a human non-secretory myeloma is fused with a human cell expressing an anti-IL-18 antibody.

In another aspect of the invention, recombinant antibodies are generated from single, isolated lymphocytes using a procedure referred to in the art as the selected lymphocyte antibody method (SLAM), as described in U.S. Patent No. 5,627,052, PCT Publication WO 92/02551 and Babcock, J.S. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:7843-7848. In this method, single cells secreting antibodies of interest, *e.g.*, lymphocytes derived from any one of the immunized animals described in Section I (A), are screened using an antigen-specific hemolytic plaque assay, wherein the antigen IL-18, or a fragment thereof, is coupled to sheep red blood cells using a linker, such as biotin, and used to identify single cells that secrete antibodies with specificity for IL-18. Following identification of antibody-secreting cells of interest, heavy- and light-chain variable region cDNAs are rescued from the cells by reverse transcriptase-PCR and these variable regions can then be expressed, in the context of appropriate immunoglobulin constant regions (*e.g.*, human constant regions), in mammalian host cells, such as COS or CHO cells. The host cells transfected with the amplified immunoglobulin sequences, derived from *in vivo* selected lymphocytes, can then undergo further analysis and selection *in vitro*, for example by panning the transfected cells to isolate cells expressing antibodies to IL-18. The amplified immunoglobulin sequences further can be manipulated *in vitro*, such as by *in vitro* affinity maturation methods such as those described in PCT Publication WO 97/29131 and PCT Publication WO 00/56772.

In vitro methods also can be used to make the antibodies of the invention, wherein an antibody library is screened to identify an antibody having the desired binding specificity. Methods for such screening of recombinant antibody libraries are well known in the art and include methods described in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT Publication No. WO 92/18619; Dower *et al.* PCT Publication No. WO 91/17271; Winter *et al.* PCT Publication No. WO 92/20791; Markland *et al.* PCT Publication No. WO 92/15679; Breitling *et al.* PCT Publication No. WO 93/01288; McCafferty *et al.* PCT Publication No. WO 92/01047; Garrard *et al.* PCT Publication No. WO 92/09690; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; McCafferty *et al.*, *Nature* (1990) 348:552-554; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol*

Biol 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982, US patent application publication 20030186374, and PCT Publication No. WO 97/29131, the contents of each of which are incorporated herein by reference.

The recombinant antibody library may be from a subject immunized with IL-18, or a portion of IL-18. Alternatively, the recombinant antibody library may be from a naïve subject, i.e., one who has not been immunized with IL-18, such as a human antibody library from a human subject who has not been immunized with human IL-18. Antibodies of the invention are selected by screening the recombinant antibody library with the peptide comprising human IL-18 (e.g., a peptide corresponding to a portion of hIL-18) to thereby select those antibodies that recognize IL-18. Methods for conducting such screening and selection are well known in the art, such as described in the references in the preceding paragraph. To select antibodies of the invention having particular binding affinities for hIL-18, such as those that dissociate from human IL-18 with a particular k_{off} rate constant, the art-known method of surface plasmon resonance can be used to select antibodies having the desired k_{off} rate constant. To select antibodies of the invention having a particular neutralizing activity for hIL-18, such as those with a particular an IC_{50}, standard methods known in the art for assessing the inhibition of hIL-18 activity may be used.

In one aspect, the invention pertains to an isolated antibody, or an antigen-binding portion thereof, that binds human IL-18. Preferably, the antibody is a neutralizing antibody. Preferably, the antibody is a human antibody. In various embodiments, the antibody is a recombinant antibody or a monoclonal antibody. The most preferred neutralizing antibody of the invention is referred to herein as 2.5(E) and has VL with amino acid sequence of SEQ ID NO: 7 and VH with amino acid sequence of SEQ ID NO: 6. Most preferrably, the 2.5(E) antibody binds human IL-18 with a K_d of less than $5x10^{10}$ M (see Example 2.2.F).

Preferrably, anti-IL-18 antibodies of the present invention, such as the 2.5(E) antibody and related antibodies, exhibit a capacity to reduce or to neutralize IL-18 activity, e.g.,as assessed by any one of several in vitro and in vivo assays known in the art (e.g., see Example 3.2.F). For example, these antibodies neutralize IL-18-induced production of human interferon gamma in KG-1 cells with IC_{50} values in the range of at least about 10^{-8} M, about 10^{-9} M, or about 10^{-10} M. Further these antibodies also neutralize IL-18-induced production of human interferon gamma in the whole blood cells with IC_{50} values in the range of at least about 10^{-8} M, about 10^{-9} M, or about 10^{-10} M.

In a particularly preferred embodiment the anti-IL-18 antibody 2.5(E) binds to human IL-18 in various forms, including pro-IL-18, mature IL-18 and truncated IL-18. The antibody 2.5(E) does not specifically bind to other cytokines, such as IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-21, TNF, LT (lymphotoxin), LTα1β2, and LTα2β1. However, the antibody 2.5(E) does exhibit cross reactivity to IL-18 from other species. For example, the antibody neutralizes the activity of IL-18 from cynomolgus monkey (IC50 for cyno IL-18= 9.1E X 10^{-11} ; See Example 2.2.J1).

In one aspect, the invention pertains to 2.5(E) antibodies and functional antibody portions, 2.5(E) -related antibodies and functional antibody portions, and other human antibodies and functional antibody portions with equivalent properties to 2.5(E), such as high affinity binding to IL-18 with low dissociation kinetics and high neutralizing capacity. In prefered embodiments, the isolated antibody, or antigen-binding portion thereof, binds human IL-18, wherein the antibody, or antigen-binding portion thereof, dissociates from human IL-18 with a k_{off} rate constant of about $0.1s^{-1}$ or less, as determined by surface plasmon resonance, or which inhibits human IL-18 activity with an IC_{50} of about 1 x 10^{-6}M or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human IL-18 with a k_{off} rate constant of about 1 x $10^{-2}s^{-1}$or less, as determined by surface plasmon resonance, or may inhibit human IL-18 activity with an IC_{50} of about 1 x 10^{-7}M or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human IL-18 with a k_{off} rate constant of about 1 x $10^{-3}s^{-1}$ or less, as determined by surface plasmon resonance, or may inhibit human IL-18 activity with an IC_{50} of about 1 x 10^{-7}M or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human IL-18 with a k_{off} rate constant of about 1 x $10^{-4}s^{-1}$ or less, as determined by surface plasmon resonance, or may inhibit human IL-18 activity with an IC_{50} of about 1 x 10^{-9}M or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human IL-18 with a k_{off} rate constant of about 1 x $10^{-5}s^{-1}$ or less, as determined by surface plasmon resonance, or may inhibit human IL-18 activity with an IC_{50} of about 1 x 10^{-10}M or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human IL-18 with a k_{off} rate constant of about 1 x $10^{-5}s^{-1}$or less, as determined by surface plasmon resonance, or may inhibit human IL-18 activity with an IC_{50} of about 1 x 10^{-11}M or less.

In still another embodiment, the invention provides an isolated human antibody, or an antigen binding portion thereof, with a light chain variable region (V_L) comprising the amino acid sequence of SEQ ID NO: 7; SEQ ID NO: 9; SEQ ID NO: 11; SEQ ID NO: 13; SEQ ID NO: 15; SEQ ID NO: 17; SEQ ID NO: 19; SEQ ID NO: 21; SEQ ID NO: 23; SEQ ID NO: 25; SEQ ID NO: 27;

SEQ ID NO: 29; SEQ ID NO: 31; SEQ ID NO: 33; SEQ ID NO: 35; SEQ ID NO: 37; SEQ ID NO: 39; or SEQ ID NO: 41, and a heavy chain variable region (V_H) comprising an amino acid sequence of SEQ ID NO: 6; SEQ ID NO: 8; SEQ ID NO: 10; SEQ ID NO: 12; SEQ ID NO: 14; SEQ ID NO: 16; SEQ ID NO: 18; SEQ ID NO: 20; SEQ ID NO: 22; SEQ ID NO: 24; SEQ ID NO: 26; SEQ ID NO: 28; SEQ ID NO: 30; SEQ ID NO: 32; SEQ ID NO: 34; SEQ ID NO: 36; SEQ ID NO: 38; or SEQ ID NO: 40.

In certain embodiments, the antibody comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region. Preferably, the heavy chain constant region is an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. Furthermore, the antibody can comprise a light chain constant region, either a kappa light chain constant region or a lambda light chain constant region. Preferably, the antibody comprises a kappa light chain constant region. Alternatively, the antibody portion can be, for example, a Fab fragment or a single chain Fv fragment.

Replacements of amino acid residues in the Fc portion to alter antibody effector function are known in the art (Winter, *et al.* US PAT NOS 5,648,260; 5624821). The Fc portion of an antibody mediates several important effector functions e.g. cytokine induction, ADCC, phagocytosis, complement dependent cytotoxicity (CDC) and half-life/ clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. In still another embodiment at least one amino acid residue is replaced in the constant region of the antibody, for example the Fc region of the antibody, such that effector functions of the antibody are altered.

One embodiment provides a labeled binding protein wherein an antibody or antibody portion of the invention is derivatized or linked to another functional molecule (e.g., another peptide or protein). For example, a labeled binding protein of the invention can be derived by functionally linking an antibody or antibody portion of the invention (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate associate of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

Useful detectable agents with which an antibody or antibody portion of the invention may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

Another embodiment of the invention provides a crystallized binding protein. Preferably the invention relates to crystals of whole anti-IL-18 antibodies and fragments thereof as disclosed herein, and formulations and compositions comprising such crystals. In one embodiment the crystallized binding protein has a greater half-life in vivo than the soluble counterpart of the binding protein. In another embodiment the binding protein retains biological activity after crystallization.

Crystallized binding protein of the invention may be produced according methods known in the art and as disclosed in WO 02072636, incorporated herein by reference. (Also see Example 2.2.M)

C. Production of recombinant IL-18 antibodies

Antibodies of the present invention may be produced by any of a number of techniques known in the art. For example, expression from host cells, wherein expression vector(s) encoding the heavy and light chains is(are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R.J. Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS

cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody of this invention. Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than the antigens of interest by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Still further the invention provides a method of synthesizing a recombinant antibody of the invention by culturing a host cell of the invention in a suitable culture medium until a recombinant antibody of the invention is synthesized. The method can further comprise isolating the recombinant antibody from the culture medium.

Table 1 is a list of amino acid sequences of VH and VL regions of preferred anti-hIL-18 antibodies of the invention. In the VH region, the naturally occuring amino acid in position 1 of the

amino terminus (N-terminus) is either Glutamate (E) or Glutamine (Q). However, to generate recombinant protein with homogeneous N-termini during large-scale production of protein comprising VH region, Glutamate (E) is preferred in position 1 of the N-terminus.

Table 1 List of Amino Acid Sequences of VH and VL regions

Protein / Protein region	Sequence Identifier	Sequence 12345678901234567890
VH 2.5(E)	**SEQ ID NO.:6**	EVQLVQSGTEVKKPGESLKI SCKGSGYTVTSYWIGWVRQM PGKGLEWMGFIYPGDSETRY SPTFQGQVTISADKSFNTAF LQWSSLKASDTAMYYCARVG SGWYPYTFDIWGQGTMVTVS S
VH 2.5 CDR-H1	Residues 31-35 of SEQ ID NO.:6	SYWIG
VH 2.5 CDR-H2	Residues 50-66 of SEQ ID NO.:6	FIYPGDSETRYSPTFQG
VH 2.5 CDR-H3	Residues 99-110 of SEQ ID NO.:6	VGSGWYPYTFDI
VL 2.5(E)	**SEQ ID NO.:7**	EIVMTQSPATLSVSPGERAT LSCRASESISSNLAWYQQKP GQAPRLFIYTASTRATDIPA RFSGSGSGTEFTLTISSLQS EDFAVYYCQQYNNWPSITFG QGTRLEIKR
VL 2.5 CDR-L1	Residues 24-34 of SEQ ID NO.:7	RASESISSNLA
VL 2.5 CDR-L2	Residues 50-56 of SEQ ID NO.:7	TASTRAT
VL 2.5 CDR-L3	Residues 89-98 of SEQ ID NO.:7	QQYNNWPSIT
VH 2.13	**SEQ ID NO.:8**	QVQLQESGPGLVTPSQTLSL TCTVSGGSISSGGHYWTWIR QHPGKGLEWIGYIYYSGSTY YNPSLKSRLTISVDTSKNQF SLKLSSVAAADTAVYYCARD RGGSGSYWDYWGQGTLVTVS S
VH 2.13 CDR-H1	Residues 31-37 of SEQ ID NO.:8	SGGHYWT
VH 2.13 CDR-H2	Residues 52-67 of SEQ ID NO.:8	YIYYSGSTYYNPSLKS
VH 2.13 CDR-H3	Residues 100-110 of SEQ ID NO.:8	DRGGSGSYWDY
VL 2.13	**SEQ ID NO.:9**	EIVLTQSPGTLSLSPGERAT LSCRGSRSVSSGYLAWYQQK PGQAPRLLIYGVSIRATGIP DRFSGSGSGTDFTLTISRLE PEDFAVYYCQQYHGSPLTFG GGTKVEIKR
VL 2.13 CDR-L1	Residues 24-35 of SEQ ID NO.:9	RGSRSVSSGYLA
VL 2.13 CDR-L2	Residues 21-27 of SEQ ID NO.:9	GVSIRAT
VL 2.13 CDR-L3	Residues 90- 98 of SEQ ID NO.:9	QQYHGSPLT

Protein / Protein region	Sequence Identifier	Sequence 12345678901234567890
VH 2.3	**SEQ ID NO.:10**	QVQLQESGPGLVKPSETLSL TCTVSGGSIRNYYWSWIRQP PGKGLEWVGYIYSSGSTNYN PSLKSRVTISVDTSKNQFSL KLSSVTAADTAVYYCARDRG GASFFDYWGQGTLVTVSS
VH 2.3 CDR-H1	Residues 31-35 of SEQ ID NO.:10	NYYWS
VH 2.3 CDR-H2	Residues 50-65 of SEQ ID NO.:10	YIYSSGSTNYNPSLKS
VH 2.3 CDR-H3	Residues 98-107 of SEQ ID NO.:10	DRGGASFFDY
VL 2.3	**SEQ ID NO.:11**	DIQMTQSPSSLSASIGDRVT ITCRASQIIGGYLNWYQQRP GKAPKFLIYSTSILQSGVPS RFSGSGSGTDFTLTISSLQP EDFATYYCQQTYITPPTFGP GTKVDIKR
VL 2.3 CDR-L1	Residues 24-34 of SEQ ID NO.:11	RASQIIGGYLN
VL 2.3 CDR-L2	Residues 50-56 of SEQ ID NO.:11	STSILQS
VL 2.3 CDR-L3	Residues 89-97 of SEQ ID NO.:11	QQTYITPPT
VH 215	**SEQ ID NO.:12**	QVQLQESGPGLVKPSQTLSL TCTVSGGSINSGDYYWSWIR QHPGKGLEWIGHISYRGTTY YNPSLKSRVTISVDTSKNQF SLKLSSVTAADTAVYCCARD RGGGFFDLWGRGTLVTVSS
VH 215 CDR-H1	Residues 31-37 of SEQ ID NO.:12	SGDYYWS
VH 215 CDR-H2	Residues 52-67 of SEQ ID NO.:12	HISYRGTTYYNPSLKS
VH 215 CDR-H3	Residues 100-108 of SEQ ID NO.:12	DRGGGFFDL
VL 215	**SEQ ID NO.:13**	EIVLTQSPGTLSLSPGERAT LSCRASRSLSSGYLAWYQQK PGQAPRLLIYGASIRATGIP DRFSGSGSATDFTLTISRLE PEDFAVYYCQQYNYSPLTFG GGTRVEINR
VL 215 CDR-L1	Residues 24-35 of SEQ ID NO.:13	RASRSLSSGYLA
VL 215 CDR-L2	Residues 51-57 of SEQ ID NO.:13	GASIRAT
VL 215 CDR-L3	Residues 90-98 of SEQ ID NO.:13	QQYNYSPLT
VH 231	**SEQ ID NO.:14**	EVQLVESGGGSVQPRGSLRL SCAASGFTFSSYSMNWVRQA PGKGLEWVSYFSSSGGIIYY ADSVKGRFTISRDNAKNSLY LQMNSLRDEDTAVYYCARDD SSGYYPYFFDYWGQGTLVTV SS
VH 231 CDR-H1	Residues 31-35 of SEQ ID NO.:14	SYSMN
VH 231 CDR-H2	Residues 50-66 of SEQ ID NO.:14	YFSSSGGIIYYADSVKG
VH 231 CDR-H3	Residues 99-111 of SEQ ID NO.:14	DDSSGYYPYFFDY

Protein / Protein region	Sequence Identifier	Sequence 12345678901234567890
VL 231	**SEQ ID NO.:15**	DIVMTQSPDSLAVSLGERAT INCKSSQTVLYRSNNKNYLA WYQQKSGQPPKLLIYWASTR ESGVPDRFSGSGSGTDFTLT ISSLQAEDVAVYYCQQYYST PLTFGGGTKVEIKR
VL 231 CDR-L1	Residues 24-40 of SEQ ID NO.:15	KSSQTVLYRSNNKNYLA
VL 231 CDR-L2	Residues 56-62 of SEQ ID NO.:15	WASTRES
VL 231 CDR-L3	Residues 95-103 of SEQ ID NO.:15	QQYYSTPLT
VH 251	**SEQ ID NO.:16**	QLQLQESGPGLVKPSETLSL TCTVSGGSISSRVYYWGWIR QPPGKGLEWIGSIYYSGSTY YNPSLKSRVTISVDASKNQF SLKLSSVTAADTAIYYCARE DSSAWVFEHWGQGTLVTVSS
VH 251 CDR-H1	Residues 31-37 of SEQ ID NO.:16	SRVYYWG
VH 251 CDR-H2	Residues 52-67 of SEQ ID NO.:16	SIYYSGSTYYNPSLKS
VH 251 CDR-H3	Residues 100-109 of SEQ ID NO.:16	EDSSAWVFEH
VL 251	**SEQ ID NO.:17**	EIVLTQSPDTLSLSPGERAT LSCRASHILSRNYLAWYQQK PGQAPRLLMYGISIRATGIP DRFSGSGSGADFTLTINRLE PEDFAVYYCQHYDNSLCSFG QGTKLEVKR
VL 251 CDR-L1	Residues 24-35 of SEQ ID NO.:17	RASHILSRNYLA
VL 251 CDR-L2	Residues 51-57 of SEQ ID NO.:17	GISIRAT
VL 251 CDR-L3	Residues 90-98 of SEQ ID NO.:17	QHYDNSLCS
VH 268	**SEQ ID NO.:18**	QVQLVESGGGVVQPGRSLRL SCAASGFTFRNYGLHWVRQA PGKGLEWVAVIWYDGSNKYY ADSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYCARES YYYYGMDVWGQGTTVTVSS
VH 268 CDR-H1	Residues 31-35 of SEQ ID NO.:18	NYGLH
VH 268 CDR-H2	Residues 20-36 of SEQ ID NO.:18	VIWYDGSNKYYADSVKG
VH 268 CDR-H3	Residues 99-108 of SEQ ID NO.:18	ESYYYYGMDV
VL 268	**SEQ ID NO.:19**	EIVMTQSPATLSVSPGERAT LSCRASQSFNSNLVWYQQKP GQAPRLLIYGASTRATGIPA RFSGSGSGTEFTLTISSLQS EDFAVYYCQQYNNWTWTFGQ GTKVEIKR
VL 268 CDR-L1	Residues 24-34 of SEQ ID NO.:19	RASQSFNSNLV
VL 268 CDR-L2	Residues 50-56 of SEQ ID NO.:19	GASTRAT
VL 268 CDR-L3	Residues 89-97 of SEQ ID NO.:19	QQYNNWTWT

Protein / Protein region	Sequence Identifier	Sequence 12345678901234567890
VH 336	**SEQ ID NO.:20**	QVQLQESGPGLVKPSQTLSL TCTVSGGSINSGDYYWSWIR QHPGKGLEWIGHISYRGTTY YNPSLKSRVTISVDTSKNQF SLKLSSVTAADTAVYCCARD RGGGFFDLWGRGTLVTVSS
VH 336 CDR-H1	Residues 31-35 of SEQ ID NO.:20	SGDYYWS
VH 336 CDR-H2	Residues 52-67 of SEQ ID NO.:20	HISYRGTTYYNPSLKS
VH 336 CDR-H3	Residues 100-108 of SEQ ID NO.:20	DRGGGFFDL
VL 336	**SEQ ID NO.:21**	EIVLTQSPGTLSLSPGERAT LSCRASQSVSSGYLAWYQQK PGQAPRLLIYGASIRATGIP DRFSGSGSATDFTLTISRLE PEDFAVYYCQQYGYSPLTFG GGTRVEINR
VL 336 CDR-L1	Residues 24-35 of SEQ ID NO.:21	RASQSVSSGYLA
VL 336 CDR-L2	Residues 51-57 of SEQ ID NO.:21	GASIRAT
VL 336 CDR-L3	Residues 90-98 of SEQ ID NO.:21	QQYGYSPLT
VH 351	**SEQ ID NO.:22**	QVQLVESGGGVVQPGRSLRL SCAASGFTFSHYGMHWVRQA PGKGLEWVAVISYDGRNKYY VDSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVFYCAREK GGSGWPPFYYYYGMDVWGQG TTVTVSS
VH 351 CDR-H1	Residues 31-35 of SEQ ID NO.:22	HYGMH
VH 351 CDR-H2	Residues 50-66 of SEQ ID NO.:22	VISYDGRNKYYVDSVKG
VH 351 CDR-H3	Residues 99-116 of SEQ ID NO.:22	EKGGSGWPPFYYYYGMDV
VL 351	**SEQ ID NO.:23**	DIVMTQTPLSLSVTPGQPAS ISCKSSQNLLYSDGETYLCW YLQKPGQPPQLLIYEVSNRF SGVPERFSGSGSGTDFTLKI SRVEAEDVGIYYCMQNVQLP LTFGGGTRVEIKR
VL 351 CDR-L1	Residues 24-39 of SEQ ID NO.:23	KSSQNLLYSDGETYLC
VL 351 CDR-L2	Residues 55-61 of SEQ ID NO.:23	EVSNRFS
VL 351 CDR-L3	Residues 94-102 of SEQ ID NO.:23	MQNVQLPLT
VH 413	**SEQ ID NO.:24**	QTQLQESGPGLVKPSETLSL TCTVSGGSISSRVYYWGWIR QPPGKGLEWIGSIYYSGSTY YSPSLKSRVTISVDTSKNQF SLKLSSVTAADTAIYYCARE DSSAWVFEHWGQGTLVTVSS
VH 413 CDR-H1	Residues 31-37 of SEQ ID NO.:24	SRVYYWG
VH 413 CDR-H2	Residues 52-67 of SEQ ID NO.:24	SIYYSGSTYYSPSLKS
VH 413 CDR-H3	Residues 100-109 of SEQ ID NO.:24	EDSSAWVFEH

Protein / Protein region	Sequence Identifier	Sequence 12345678901234567890
VL 413	SEQ ID NO.:25	EIVLTQSPDTLSLSPGERAT LSCRASQILSRNYLAWYQQK PGQAPRLLIYGISIRATGIP DRFSGSGSGADFTLTINRLE PEDFAVYYCQHYDNSLCSFG QGTKLEVKR
VL 413 CDR-L1	Residues 24-35 of SEQ ID NO.:25	RASQILSRNYLA
VL 413 CDR-L2	Residues 51-57 of SEQ ID NO.:25	GISIRAT
VL 413 CDR-L3	Residues 90-98 of SEQ ID NO.:25	QHYDNSLCS
VH 435	SEQ ID NO.:26	QLQLQESGPGLVKPSETLSL TCTVSGGSIDSRIYYWGWIR QPPGKGLEWIGSIYYRGSTY YNPSLKSRVTISVDTPKNQF SLKLNSVTAADTAVYYCARE DSSAWVFDYWGQGTLATVSS
VH 435 CDR-H1	Residues 31-37 of SEQ ID NO.:26	SRIYYWG
VH 435 CDR-H2	Residues 52-67 of SEQ ID NO.:26	SIYYRGSTYYNPSLKS
VH 435 CDR-H3	Residues 100-109 of SEQ ID NO.:26	EDSSAWVFDY
VL 435	SEQ ID NO.:27	EIVLTQSPGTLSLSPGERAT LSCRASQSVRNNYLNWYQQK PGQAPRLLIYGAFSRATGIP DRFSGSGSGTDFTLTISSLE PEDFVVYYCQQYGNSIDSFG QGTKLEINR
VL 435 CDR-L1	Residues 24-35 of SEQ ID NO.:27	RASQSVRNNYLN
VL 435 CDR-L2	Residues 51-57 of SEQ ID NO.:27	GAFSRAT
VL 435 CDR-L3	Residues 90-98 of SEQ ID NO.:27	QYGNSIDS
VH 444	SEQ ID NO.:28	QVQLQESGPGLVKPSQTLSL TCTVSGGSINSGDYYWSYIR QHPGKGLEWIGHISYRGTTY YNPSLKSRVTISVDTSKNQF SLKLSSVTAADTAVYCCARD RGGGFFDLWGRGTLVTVSS
VH 444 CDR-H1	Residues 31-37 of SEQ ID NO.:28	SGDYYWS
VH 444 CDR-H2	Residues 52-67 of SEQ ID NO.:28	HISYRGTTYYNPSLKS
VH 444 CDR-H3	Residues 100-108 of SEQ ID NO.:28	DRGGGFFDL
VL 444	SEQ ID NO.:29	EIVLTQSPGTLSLSPGERAT LSCRASQSVSSGYLAWYQRK PGQAPRLLIYGTSIRATGIP DRFSGSGSATDFTLSISRLG PEDFAVYYCQQYGYSPLTFG GGTRVEINR
VL 444 CDR-L1	Residues 24-35 of SEQ ID NO.:29	RASQSVSSGYLA
VL 444 CDR-L2	Residues 51-57 of SEQ ID NO.:29	GTSIRAT
VL 444 CDR-L3	Residues 90-98 of SEQ ID NO.:29	QQYGYSPLT

Protein / Protein region	Sequence Identifier	Sequence 12345678901234567890
VH 478	**SEQ ID NO.:30**	QVQLQESGPGLVKPSQTLSL TCTVSGGSISSGGHYWSWIR QHPGKGLEWIGYIYYSGSTH YNPSLKSRVTISVDTSKNQF SLKLRSVSAADTAGYYCASL YNGNGYFDLWGRGTLVTVSS
VH 478 CDR-H1	Residues 31-37 of SEQ ID NO.:30	SGGHYWS
VH 478 CDR-H2	Residues 52-67 of SEQ ID NO.:30	YIYYSGSTHYNPSLKS
VH 478 CDR-H3	Residues 99-109 of SEQ ID NO.:30	SLYNGNGYFDL
VL 478	**SEQ ID NO.:31**	EIVLTQSPGTLSLSPGERAT LSCRASQSISSGYLAWYQQK PGQAPRLIIYGVSRRATGIP DRFSGSGSGADFTLTISRLD PEDFVVYYCQQYGFSPLTFG GGTKVEIKR
VL 478 CDR-L1	Residues 24-35 of SEQ ID NO.:31	RASQSISSGYLA
VL 478 CDR-L2	Residues 51-57 of SEQ ID NO.:31	GVSRRAT
VL 478 CDR-L3	Residues 90-98 of SEQ ID NO.:31	QQYGFSPLT
VH 521	**SEQ ID NO.:32**	QLQLQESGPGLVKPSETLSL TCTVSGGSISRSYDYWGWIR QPPGKGLEWIGSIYYRGSTY YNPSLKSRVTISVDTSKNQF SLKLSSVTAADTAVYYCARE YSTTWSIDYWGQGTLVTVSS
VH 521 CDR-H1	Residues 31-37 of SEQ ID NO.:32	RSYDYWG
VH 521 CDR-H2	Residues 52-67 of SEQ ID NO.:32	SIYYRGSTYYNPSLKS
VH 521 CDR-H3	Residues 100-109 of SEQ ID NO.:32	EYSTTWSIDY
VL 521	**SEQ ID NO.:33**	ENVLTQSPGTLSLSPGERAT LSCRASQSIRNNYLAWYQQK PGQAPRLLIHGASSRATGIP DRFGGSGSGTDFTLTISRLE PEDFAVYFCQQYGNSIITFG PGTKVDVNR
VL 521 CDR-L1	Residues 24-35 of SEQ ID NO.:33	RASQSIRNNYLA
VL 521 CDR-L2	Residues 51-57 of SEQ ID NO.:33	GASSRAT
VL 521 CDR-L3	Residues 90-98 of SEQ ID NO.:33	QQYGNSIIT
VH 550	**SEQ ID NO.:34**	QVQLQESGPGLVKPSQTLSL TCTVSGGSINSGGYYWSWIR QHPGKGLEWIGHISYRGTTY SNPSLKSRVTISVDTSKNQF SLKLSSVTAADTAVYYCARD RGGGFFDLWGRGTLVTVSS
VH 550 CDR-H1	Residues 31-37 of SEQ ID NO.:34	SGGYYWS
VH 550 CDR-H2	Residues 52-67 of SEQ ID NO.:34	HISYRGTTYSNPSLKS
VH 550 CDR-H3	Residues 100-108 of SEQ ID NO.:34	DRGGGFFDL

Protein / Protein region	Sequence Identifier	Sequence 12345678901234567890
VL 550	**SEQ ID NO.:35**	EIVLTQSPGTLSLSPGERAT LSCRASQSVNSGYLAWYQQK PGQAPRLLIYGVSIRATDIP DRFSGSGSATDFTLTISRLE PEDFAVYYCQQYGFSPLTFG GGTRVEINR
VL 550 CDR-L1	Residues 24-35 of SEQ ID NO.:35	RASQSVNSGYLA
VL 550 CDR-L2	Residues 51-57 of SEQ ID NO.:35	GVSIRAT
VL 550 CDR-L3	Residues 90-98 of SEQ ID NO.:35	QQYGFSPLT
VH 581	**SEQ ID NO.:36**	QVQLVESGGGVVQPGRSLRL SCAASGFTFSHCGMHWVRQA PGKGLEWVAVISYDGSNKYY ADSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYCAKDH GGSGSPPFYYYYGMDVWGQG TTVTVSS
VH 581 CDR-H1	Residues 31-35 of SEQ ID NO.:36	HCGMH
VH 581 CDR-H2	Residues 50-66 of SEQ ID NO.:36	VISYDGSNKYYADSVKG
VH 581 CDR-H3	Residues 99-116 of SEQ ID NO.:36	DHGGSGSPPFYYYYGMDV
VL 581	**SEQ ID NO.:37**	DILMTQTPLSLSVTPGQPAS ISCKSSQSLLHGDGKTYLYW YLQKPGQPPQFLIQELSNRF SGVPDRFSGSGSGTDFTLKI SRXEAEDVGVYYCMQSLQLP LTFGGGTKVQIKR
VL 581 CDR-L1	Residues 24-39 of SEQ ID NO.:37	KSSQSLLHGDGKTYLY
VL 581 CDR-L2	Residues 55-61 of SEQ ID NO.:37	ELSNRFS
VL 581 CDR-L3	Residues 94-102 of SEQ ID NO.:37	MQSLQLPLT
VH 7.5	**SEQ ID NO.:38**	QVQLVESGGGVVQPGRSLRL SCAASGFTFSYYGMHWVRQA PGKGLEWVAVIWYDGRNKYY ADSVKGRVTISRDNSKKTLY LQMNSLRAEDTAVYYCAREG GYYYGMDVWGQGTTVTVSS
VH 7.5 CDR-H1	Residues 31-35 of SEQ ID NO.:38	YYGMH
VH 7.5 CDR-H2	Residues 50-66 of SEQ ID NO.:38	VIWYDGRNKYYADSVKG
VH 7.5 CDR-H3	Residues 99-108 of SEQ ID NO.:38	EGGYYYGMDV
VL 7.5	**SEQ ID NO.:39**	EILLTQSPGTLSLSPGERAT LSCRASQNVSSSYLAWYQQN PGQAPRLLIYGASSRATGIP DRFSGSGSGTDFTLTISRLE PEDFEVYYCQQSGSSLFTFG PGTKVDIKR
VL 7.5 CDR-L1	Residues 24-35 of SEQ ID NO.:39	RASQNVSSSYLA
VL 7.5 CDR-L2	Residues 51-57 of SEQ ID NO.:39	GASSRAT
VL 7.5 CDR-L3	Residues 90-98 of SEQ ID NO.:39	QQSGSSLFT

Protein / Protein region	Sequence Identifier	Sequence 12345678901234567890
VH 2.11	SEQ ID NO.:40	QVQLQESGPGLVKPSQTLSL TCTVSGGSIRSGDHYWTWIR QHPGKGLEWIGHIYYSGSTY YNPSLKSRLTISIDTSKNQF SLKLSSVTAADTAVYYCARD YGGNGYFDYWGQGTLVTVSS
VH 2.11 CDR-H1	Residues 31-37 of SEQ ID NO.:40	SGDHYWT
VH 2.11 CDR-H2	Residues 52-67 of SEQ ID NO.:40	HIYYSGSTYYNPSLKS
VH 2.11 CDR-H3	Residues 97-109 of SEQ ID NO.:40	CARDYGGNGYFDY
VL 2.11	SEQ ID NO.:41	DIVMTQTPLSLPVTPGEPAS ISCRSSQSLLDSDDGNTYLD WYLQKPGQSPQLLIYTLSYR ASGVPDRFSGSGSGTDFTLN ISRVEAEDVGVYYCMQRIEF PITFGQGTRLEIKR
VL 2.11 CDR-L1	Residues 24-40 of SEQ ID NO.:41	RSSQSLLDSDDGNTYLD
VL 2.11 CDR-L2	Residues 56-62 of SEQ ID NO.:41	TLSYRAS
VL 2.11 CDR-L3	Residues 95-103 of SEQ ID NO.:41	MQRIEFPIT

The foregoing isolated anti-IL-18 antibody CDR sequences establish a novel family of IL-18 binding proteins, isolated in accordance with this invention, and comprising polypeptides that include the CDR sequences listed in Table 2 below. To generate and to select CDR's of the invention having preferred IL-18 binding and/or neutralizing activity, standard methods known in the art for generating binding proteins of the present invention and assessing the IL-18 binding and/or neutralizing characteristics of those binding protein may be used, including but not limited to those specifically described herein.

Table 2: Consensus IL-18 CDR affinity ligands (alternative residues are listed below each amino acid position; - indicates residue may be absent).

CDR region	Sequence Identifier	Consensus Sequence
CDR-H1	SEQ ID NO.:42	X_1 X_2 X_3 X_4 X_5 X_6 X_7 S Y W I G - - N G G H Y W T H R Y W S S G R S D Y N H Y C S M V L I D
CDR-H2	SEQ ID NO.:43	X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13} X_{14} X_{15} X_{16} X_{17} F I Y P G D S E T R Y S P P T F Q - Y F S Y S G T T Y Y N S A D L K S G H W S R G R I N I S V S K S D K H V
CDR-H3	SEQ ID NO.:44	X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13} X_{14} X_{15} X_{16} X_{17} X_{18} V G S G W Y P Y T - - - - - - - - - - D R G S S G S F D W F D Y I Y G M D V E D Y Y A S F D L D Y F D Y S K R N G F P M Y H F Y C L T D Y W I E V L Y N P Y A G F W V
CDR-L1	SEQ ID NO.:45	X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13} X_{14} X_{15} X_{16} X_{17} R A S E S I S S N L A - - - - - - K G R I V G G G G Y L N A K N Y L C A S Q T L L Y Y R S S V N E T Y C D H N F N R R N D G K N H D
CDR-L2	SEQ ID NO.:46	X_1 X_2 X_3 X_4 X_5 X_6 X_7 T A S T R A T G V F I L Q S S T N S E W I R F E L Y
CDR-L3	SEQ ID NO.:47	X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} Q Q Y N N W P S - - M H N H G S L L I T Y G Y G I T L P S S D Y L I C W R G S I F I V Q F F L E I

D. Uses of Anti-IL-18 Antibodies

Given their ability to bind to human IL-18, the anti-human IL-18 antibodies, or portions thereof, of the invention can be used to detect human IL-18 (*e.g.*, in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. The invention provides a method for detecting human IL-18 in a biological sample comprising contacting a biological sample with an antibody, or antibody portion, of the invention and detecting either the antibody (or antibody portion) bound to human IL-18 or unbound antibody (or antibody portion), to thereby detect human IL-18 in the biological sample. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm.

Alternative to labeling the antibody, human IL-18 can be assayed in biological fluids by a competition immunoassay utilizing rhIL-18 standards labeled with a detectable substance and an unlabeled anti- human IL-18 antibody. In this assay, the biological sample, the labeled rhIL-18 standards and the anti- human IL-18 antibody are combined and the amount of labeled rhIL-18 standard bound to the unlabeled antibody is determined. The amount of human IL-18 in the biological sample is inversely proportional to the amount of labeled rhIL-18 standard bound to the anti-IL-18 antibody.

The antibodies and antibody portions of the invention preferably are capable of neutralizing human IL-18 activity both *in vitro* and *in vivo*. Accordingly, such antibodies and antibody portions of the invention can be used to inhibit hIL-18 activity, *e.g.*, in a cell culture containing hIL-18, in human subjects or in other mammalian subjects having IL-18 with which an antibody of the invention cross-reacts. In one embodiment, the invention provides a method for inhibiting IL-18 activity comprising contacting IL-18 with an antibody or antibody portion of the invention such that IL-18 activity is inhibited. Preferably, the IL-18 is human IL-18. For example, in a cell culture

containing, or suspected of containing hIL-18, an antibody or antibody portion of the invention can be added to the culture medium to inhibit hIL-18 activity in the culture.

In another embodiment, the invention provides a method for reducing IL-18 activity in a subject, advantageously from a subject suffering from a disease or disorder in which IL-18 activity is detrimental. The invention provides methods for reducing IL-18 activity in a subject suffering from such a disease or disorder, which method comprises administering to the subject an antibody or antibody portion of the invention such that IL-18 activity in the subject is reduced. Preferably, the IL-18 is human IL-18 and the subject is a human subject. Alternatively, the subject can be a mammal expressing an IL-18 to which an antibody of the invention is capable of binding. Still further the subject can be a mammal into which hIL-18 has been introduced (*e.g.*, by administration of hIL-18 or by expression of an hIL-18 transgene). An antibody of the invention can be administered to a human subject for therapeutic purposes. Moreover, an antibody of the invention can be administered to a non-human mammal expressing an IL-18 with which the antibody is capable of binding for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (*e.g.*, testing of dosages and time courses of administration).

As used herein, the term "a disorder in which IL-18 activity is detrimental" is intended to include diseases and other disorders in which the presence of IL-18 in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which IL-18 activity is detrimental is a disorder in which reduction of IL-18 activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of IL-18 in a biological fluid of a subject suffering from the disorder (*e.g.*, an increase in the concentration of IL-18 in serum, plasma, synovial fluid, *etc.* of the subject), which can be detected, for example, using an anti-IL-18 antibody as described above. Non-limiting examples of disorders that can be treated with the antibodies of the invention include those disorders discussed in the section below pertaining to pharmaceutical compositions of the antibodies of the invention.

D. Pharmaceutical Composition

The invention also provides pharmaceutical compositions comprising an antibody, or antigen-binding portion thereof, of the invention and a pharmaceutically acceptable carrier. In one

embodiment, the pharmaceutical composition further comprises at least one additional therapeutic agent for treating a disorder in which IL-18 activity is detrimental.

The antibodies and antibody-portions of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody or antibody portion of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

The antibodies and antibody-portions of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. Preferably, the antibody or antibody-portions will be prepared as an injectable solution containing 0.1-250 mg/ml antibody. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants.

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical

preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (*i.e.*, antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including, in the composition, an agent that delays absorption, for example, monostearate salts and gelatin.

The antibodies and antibody-portions of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous injection, intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, an antibody or antibody portion of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and

other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an antibody or antibody portion of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating disorders in which IL-18 activity is detrimental. For example, an anti-hIL-18 antibody or antibody portion of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (*e.g.*, antibodies that bind other cytokines or that bind cell surface molecules). Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

In certain embodiments, an antibody to IL-18 or fragment thereof is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Such vehicles are described, e.g., in U.S. Application Serial No. 09/428,082 and published PCT Application No. WO 99/25044, which are hereby incorporated by reference for any purpose.

Interleukin 18 plays a critical role in the pathology associated with a variety of diseases involving immune and inflammatory elements. These diseases include, but are not limited to, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's

disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthopathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthopathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasulitis of the kidneys, lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjörgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic

thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatatis, idiosyncratic liver disease, Drug-Induced hepatitis, Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (*e.g.*, depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma). The human antibodies, and antibody portions of the invention can be used to treat humans suffering from autoimmune diseases, in particular those associated with inflammation, including, rheumatoid spondylitis, allergy, autoimmune diabetes, autoimmune uveitis.

Preferably, the antibodies of the invention or antigen-binding portions thereof, are used to treat rheumatoid arthritis, Crohn's disease, multiple sclerosis, insulin dependent diabetes, mellitus and psoriasis.

An antibody, or antibody portion, of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of autoimmune and inflammatory diseases.

Antibodies of the invention, or antigen binding portions thereof can be used alone or in combination to treat such diseases. It should be understood that the antibodies of the invention or antigen binding portion thereof can be used alone or in combination with an additional agent, e.g., a therapeutic agent, said additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the antibody of the present invention. The additional agent also can be an agent that imparts a beneficial attribute to the therapeutic composition e.g., an agent which effects the viscosity of the composition.

It should further be understood that the combinations which are to be included within this invention are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations, which are part of this invention, can be the antibodies of the present invention and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

Preferred combinations are non-steroidal anti-inflammatory drug(s) also referred to as NSAIDS which include drugs like ibuprofen. Other preferred combinations are corticosteroids

including prednisolone; the well known side-effects of steroid use can be reduced or even eliminated by tapering the steroid dose required when treating patients in combination with the anti-IL-18 antibodies of this invention. Non-limiting examples of therapeutic agents for rheumatoid arthritis with which an antibody, or antibody portion, of the invention can be combined include the following: cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-12, IL-15, IL-16, IL-21, IL-23, interferons, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, CTLA or their ligands including CD154 (gp39 or CD40L).

Preferred combinations of therapeutic agents may interfere at different points in the autoimmune and subsequent inflammatory cascade; preferred examples include TNF antagonists like chimeric, humanized or human TNF antibodies, D2E7, (PCT Publication No. WO 97/29131), CA2 (Remicade™), CDP 571, and soluble p55 or p75 TNF receptors, derivatives, thereof, (p75TNFR1gG (Enbrel™) or p55TNFR1gG (Lenercept), and also TNFα converting enzyme (TACE) inhibitors; similarly IL-1 inhibitors (Interleukin-1-converting enzyme inhibitors, IL-1RA etc.) may be effective for the same reason. Other preferred combinations include Interleukin 11. Yet another preferred combination are other key players of the autoimmune response which may act parallel to, dependent on or in concert with IL-18 function; especially preferred are IL-12 antagonists including IL-12 antibodies or soluble IL-12 receptors, or IL-12 binding proteins. It has been shown that IL-12 and IL-18 have overlapping but distinct functions and a combination of antagonists to both may be most effective. Yet another preferred combination are non-depleting anti-CD4 inhibitors. Yet other preferred combinations include antagonists of the co-stimulatory pathway CD80 (B7.1) or CD86 (B7.2) including antibodies, soluble receptors or antagonistic ligands.

The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine chloroquinine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, cochicine, corticosteroids (oral, inhaled and local injection), beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral), xanthines (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium and oxitropium, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adensosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as

TNFα or IL-1 (e.g. IRAK, NIK, IKK , p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TNFα converting enzyme (TACE) inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors and the derivatives p75TNFRIgG (Enbrel™ and p55TNFRIgG (Lenercept)), sIL-1RI, sIL-1RII, sIL-6R), antiinflammatory cytokines (e.g. IL-4, IL-10, IL-11, IL-13 and TGFβ), celecoxib, folic acid, hydroxychloroquine sulfate, rofecoxib, etanercept, infliximab, naproxen, valdecoxib, sulfasalazine, methylprednisolone, meloxicam, methylprednisolone acetate, gold sodium thiomalate, aspirin, triamcinolone acetonide, propoxyphene napsylate/apap, folate, nabumetone, diclofenac, piroxicam, etodolac, diclofenac sodium, oxaprozin, oxycodone hcl, hydrocodone bitartrate/apap, diclofenac sodium/misoprostol, fentanyl, anakinra, human recombinant, tramadol hcl, salsalate, sulindac, cyanocobalamin/fa/pyridoxine, acetaminophen, alendronate sodium, prednisolone, morphine sulfate, lidocaine hydrochloride, indomethacin, glucosamine sulf/chondroitin, amitriptyline hcl, sulfadiazine, oxycodone hcl/acetaminophen, olopatadine hcl, misoprostol, naproxen sodium, omeprazole, cyclophosphamide, rituximab, IL-1 TRAP, MRA, CTLA4-IG, IL-18 BP, anti-IL-12, Anti-IL15, BIRB-796, SCIO-469, VX-702, AMG-548, VX-740, Roflumilast, IC-485, CDC-801, and Mesopram. Preferred combinations include methotrexate or leflunomide and in moderate or severe rheumatoid arthritis cases, cyclosporine.

Non-limiting examples of therapeutic agents for inflammatory bowel disease with which an antibody, or antibody portion, of the invention can be combined include the following: budenoside; epidermal growth factor; corticosteroids; cyclosporin, sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxygenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1β monoclonal antibodies; anti-IL-6 monoclonal antibodies; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-12, IL-15, IL-16, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands. The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as

TNFα or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TNFα converting enzyme inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R) and antiinflammatory cytokines (e.g. IL-4, IL-10, IL-11, IL-13 and TGFβ).

Preferred examples of therapeutic agents for Crohn's disease in which an antibody or an antigen binding portion can be combined include the following: TNF antagonists, for example, anti-TNF antibodies, D2E7 (PCT Publication No. WO 97/29131; HUMIRA), CA2 (REMICADE), CDP 571, TNFR-Ig constructs, (p75TNFRIgG (ENBREL) and p55TNFRIgG (LENERCEPT)) inhibitors and PDE4 inhibitors. Antibodies of the invention, or antigen binding portions thereof, can be combined with corticosteroids, for example, budenoside and dexamethasone. Antibodies of the invention or antigen binding portions thereof, may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid and olsalazine, and agents which interfere with synthesis or action of proinflammatory cytokines such as IL-1, for example, IL-1β converting enzyme inhibitors and IL-1ra. Antibodies of the invention or antigen binding portion thereof may also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors 6-mercaptopurines. Antibodies of the invention, or antigen binding portions thereof, can be combined with IL-11. Antibodies of the invention, or antigen binding portions thereof, can be combined with mesalamine, prednisone, azathioprine, mercaptopurine, infliximab, methylprednisolone sodium succinate, diphenoxylate/atrop sulfate, loperamide hydrochloride, methotrexate, omeprazole, folate, ciprofloxacin/dextrose-water, hydrocodone bitartrate/apap, tetracycline hydrochloride, fluocinonide, metronidazole, thimerosal/boric acid, cholestyramine/sucrose, ciprofloxacin hydrochloride, hyoscyamine sulfate, meperidine hydrochloride, midazolam hydrochloride, oxycodone hcl/acetaminophen, promethazine hydrochloride, sodium phosphate, sulfamethoxazole/trimethoprim, celecoxib, polycarbophil, propoxyphene napsylate, hydrocortisone, multivitamins, balsalazide disodium, codeine phosphate/apap, colesevelam hcl, cyanocobalamin, folic acid, levofloxacin, methylprednisolone, natalizumab and interferon-gamma

Non-limiting examples of therapeutic agents for multiple sclerosis with which an antibody, or antibody portion, of the invention can be combined include the following: corticosteroids; prednisolone; methylprednisolone; azathioprine; cyclophosphamide; cyclosporine; methotrexate; 4-aminopyridine; tizanidine; interferon-β1a (AVONEX; Biogen); interferon-β1b (BETASERON; Chiron/Berlex); interferon α-n3) (Interferon Sciences/Fujimoto), interferon-α (Alfa

Wassermann/J&J), interferon β1A-IF (Serono/Inhale Therapeutics), Peginterferon α 2b (Enzon/Schering-Plough), Copolymer 1 (Cop-1; COPAXONE; Teva Pharmaceutical Industries, Inc.); hyperbaric oxygen; intravenous immunoglobulin; clabribine; antibodies to or antagonists of other human cytokines or growth factors and their receptors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-12, IL-23, IL-15, IL-16, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD19, CD20, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands. The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adensosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNFα or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TACE inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R) and antiinflammatory cytokines (e.g. IL-4, IL-10, IL-13 and TGFβ).

Preferred examples of therapeutic agents for multiple sclerosis in which the antibody or antigen binding portion thereof can be combined to include interferon-β, for example, IFNβ1a and IFNβ1b; copaxone, corticosteroids, caspase inhibitors , for example inhibitors of caspase-1, IL-1 inhibitors, TNF inhibitors, and antibodies to CD40 ligand and CD80.

The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as alemtuzumab, dronabinol, Unimed,daclizumab,mitoxantrone, xaliproden hydrochloride, fampridine, glatiramer acetate, natalizumab, sinnabidol, a-immunokine NNSO3, ABR-215062, AnergiX.MS, chemokine receptor antagonists, BBR-2778, calagualine, CPI-1189, LEM (liposome encapsulated mitoxantrone), THC.CBD (cannabinoid agonist) MBP-8298, mesopram (PDE4 inhibitor), MNA-715, anti-IL-6 receptor antibody, neurovax, pirfenidone allotrap 1258 (RDP-1258), sTNF-R1, talampanel, teriflunomide,TGF-beta2, tiplimotide, VLA-4 antagonists (for example, TR-14035, VLA4 Ultrahaler, Antegran-ELAN/Biogen), interferon gamma antagonists, IL-4 agonists.

Non-limiting examples of therapeutic agents for Angina with which an antibody, or antibody portion, of the invention can be combined include the following: aspirin, nitroglycerin, isosorbide mononitrate, metoprolol succinate, atenolol, metoprolol tartrate, amlodipine besylate, diltiazem

hydrochloride, isosorbide dinitrate, clopidogrel bisulfate, nifedipine, atorvastatin calcium, potassium chloride, furosemide, simvastatin, verapamil hcl, digoxin, propranolol hydrochloride, carvedilol, lisinopril, spironolactone, hydrochlorothiazide, enalapril maleate, nadolol, ramipril, enoxaparin sodium, heparin sodium, valsartan, sotalol hydrochloride, fenofibrate, ezetimibe, bumetanide, losartan potassium, lisinopril/hydrochlorothiazide, felodipine, captopril, bisoprolol fumarate.

Non-limiting examples of therapeutic agents for Ankylosing Spondylitis with which an antibody, or antibody portion, of the invention can be combined include the following: ibuprofen, diclofenac and misoprostol, naproxen, meloxicam, indomethacin, diclofenac, celecoxib, rofecoxib, Sulfasalazine, Methotrexate, azathioprine, minocyclin, prednisone, etanercept, infliximab.

Non-limiting examples of therapeutic agents for Asthma with which an antibody, or antibody portion, of the invention can be combined include the following: albuterol, salmeterol/fluticasone, montelukast sodium, fluticasone propionate, budesonide, prednisone, salmeterol xinafoate, levalbuterol hcl, albuterol sulfate/ipratropium, prednisolone sodium phosphate, triamcinolone acetonide, beclomethasone dipropionate, ipratropium bromide, azithromycin, pirbuterol acetate, prednisolone, theophylline anhydrous, methylprednisolone sodium succinate, clarithromycin, zafirlukast, formoterol fumarate, influenza virus vaccine, methylprednisolone, amoxicillin trihydrate, flunisolide, allergy injection, cromolyn sodium, fexofenadine hydrochloride, flunisolide/menthol, amoxicillin/clavulanate, levofloxacin, inhaler assist device, guaifenesin, dexamethasone sodium phosphate, moxifloxacin hcl, doxycycline hyclate, guaifenesin/d-methorphan, p-ephedrine/cod/chlorphenir, gatifloxacin, cetirizine hydrochloride, mometasone furoate, salmeterol xinafoate, benzonatate, cephalexin, pe/hydrocodone/chlorphenir, cetirizine hcl/pseudoephed, phenylephrine/cod/promethazine, codeine/promethazine, cefprozil, dexamethasone, guaifenesin/pseudoephedrine, chlorpheniramine/hydrocodone, nedocromil sodium, terbutaline sulfate, epinephrine, methylprednisolone, metaproterenol sulfate.

Non-limiting examples of therapeutic agents for COPD with which an antibody, or antibody portion, of the invention can be combined include the following: albuterol sulfate/ipratropium, ipratropium bromide, salmeterol/fluticasone, albuterol, salmeterol xinafoate, fluticasone propionate, prednisone, theophylline anhydrous, methylprednisolone sodium succinate, montelukast sodium, budesonide, formoterol fumarate, triamcinolone acetonide, levofloxacin, guaifenesin, azithromycin, beclomethasone dipropionate, levalbuterol hcl, flunisolide, ceftriaxone sodium, amoxicillin trihydrate, gatifloxacin, zafirlukast, amoxicillin/clavulanate, flunisolide/menthol, chlorpheniramine/hydrocodone, metaproterenol sulfate, methylprednisolone, mometasone furoate, p-

ephedrine/cod/chlorphenir, pirbuterol acetate, p-ephedrine/loratadine, terbutaline sulfate, tiotropium bromide, (R,R)-formoterol, TgAAT, Cilomilast, Roflumilast.

Non-limiting examples of therapeutic agents for HCV with which an antibody, or antibody portion, of the invention can be combined include the following: Interferon-alpha-2a, Interferon-alpha-2b, Interferon-alpha con1, Interferon-alpha-n1, Pegylated interferon-alpha-2a, Pegylated interferon-alpha-2b, ribavirin, Peginterferon alfa-2b + ribavirin, Ursodeoxycholic Acid, Glycyrrhizic Acid, Thymalfasin, Maxamine, VX-497 and any compounds that are used to treat HCV through intervention with the following targets:HCV polymerase, HCV protease, HCV helicase, HCV IRES (internal ribosome entry site).

Non-limiting examples of therapeutic agents for Idiopathic Pulmonary. Fibrosis with which an antibody, or antibody portion, of the invention can be combined include the following: prednisone, azathioprine, albuterol, colchicine, albuterol sulfate, digoxin, gamma interferon, methylprednisolone sod succ, lorazepam, furosemide, lisinopril, nitroglycerin, spironolactone, cyclophosphamide, ipratropium bromide, actinomycin d, alteplase, fluticasone propionate, levofloxacin, metaproterenol sulfate, morphine sulfate, oxycodone hcl, potassium chloride, triamcinolone acetonide, tacrolimus anhydrous, calcium, interferon-alpha, methotrexate, mycophenolate mofetil, Interferon-gamma-1β.

Non-limiting examples of therapeutic agents for Myocardial Infarction with which an antibody, or antibody portion, of the invention can be combined include the following: aspirin, nitroglycerin, metoprolol tartrate, enoxaparin sodium, heparin sodium, clopidogrel bisulfate, carvedilol, atenolol, morphine sulfate, metoprolol succinate, warfarin sodium, lisinopril, isosorbide mononitrate, digoxin, furosemide, simvastatin, ramipril, tenecteplase, enalapril maleate, torsemide, retavase, losartan potassium, quinapril hcl/mag carb, bumetanide, alteplase, enalaprilat, amiodarone hydrochloride, tirofiban hcl m-hydrate, diltiazem hydrochloride, captopril, irbesartan, valsartan, propranolol hydrochloride, fosinopril sodium, lidocaine hydrochloride, eptifibatide, cefazolin sodium, atropine sulfate, aminocaproic acid, spironolactone, interferon, sotalol hydrochloride, potassium chloride, docusate sodium, dobutamine hcl, alprazolam, pravastatin sodium, atorvastatin calcium, midazolam hydrochloride, meperidine hydrochloride, isosorbide dinitrate, epinephrine, dopamine hydrochloride, bivalirudin, rosuvastatin, ezetimibe/simvastatin, avasimibe, cariporide.

Non-limiting examples of therapeutic agents for Psoriasis with which an antibody, or antibody portion, of the invention can be combined include the following: calcipotriene, clobetasol propionate, triamcinolone acetonide, halobetasol propionate, tazarotene, methotrexate, fluocinonide, betamethasone diprop augmented, fluocinolone acetonide, acitretin, tar shampoo, betamethasone

valerate, mometasone furoate, ketoconazole, pramoxine/fluocinolone, hydrocortisone valerate, flurandrenolide, urea, betamethasone, clobetasol propionate/emoll, fluticasone propionate, azithromycin, hydrocortisone, moisturizing formula, folic acid, desonide, pimecrolimus, coal tar, diflorasone diacetate, etanercept folate, lactic acid, methoxsalen, hc/bismuth subgal/znox/resor, methylprednisolone acetate, prednisone, sunscreen, halcinonide, salicylic acid, anthralin, clocortolone pivalate, coal extract, coal tar/salicylic acid, coal tar/salicylic acid/sulfur, desoximetasone, diazepam, emollient, fluocinonide/emollient, mineral oil/castor oil/na lact, mineral oil/peanut oil, petroleum/isopropyl myristate, psoralen, salicylic acid, soap/tribromsalan, thimerosal/boric acid, celecoxib, infliximab, cyclosporine, alefacept, efalizumab, tacrolimus, pimecrolimus, PUVA, UVB, sulfasalazine.

Non-limiting examples of therapeutic agents for Psoriatic Arthritis with which an antibody, or antibody portion, of the invention can be combined include the following: methotrexate, etanercept, rofecoxib, celecoxib, folic acid, sulfasalazine, naproxen, leflunomide, methylprednisolone acetate, indomethacin, hydroxychloroquine sulfate, prednisone, sulindac, betamethasone diprop augmented, infliximab, methotrexate, folate, triamcinolone acetonide, diclofenac, dimethylsulfoxide, piroxicam, diclofenac sodium, ketoprofen, meloxicam, methylprednisolone, nabumetone, tolmetin sodium, calcipotriene, cyclosporine, diclofenac sodium/misoprostol, fluocinonide, glucosamine sulfate, gold sodium thiomalate, hydrocodone bitartrate/apap, ibuprofen, risedronate sodium, sulfadiazine, thioguanine, valdecoxib, alefacept, efalizumab.

Non-limiting examples of therapeutic agents for Restenosis with which an antibody, or antibody portion, of the invention can be combined include the following: sirolimus, paclitaxel, everolimus, tacrolimus, ABT-578, acetaminophen.

Non-limiting examples of therapeutic agents for Sciatica with which an antibody, or antibody portion, of the invention can be combined include the following: hydrocodone bitartrate/apap, rofecoxib, cyclobenzaprine hcl, methylprednisolone, naproxen, ibuprofen, oxycodone hcl/acetaminophen, celecoxib, valdecoxib, methylprednisolone acetate, prednisone, codeine phosphate/apap, tramadol hcl/acetaminophen, metaxalone, meloxicam, methocarbamol, lidocaine hydrochloride, diclofenac sodium, gabapentin, dexamethasone, carisoprodol, ketorolac tromethamine, indomethacin, acetaminophen, diazepam, nabumetone, oxycodone hcl, tizanidine hcl, diclofenac sodium/misoprostol, propoxyphene napsylate/apap, asa/oxycod/oxycodone ter, ibuprofen/hydrocodone bit, tramadol hcl, etodolac, propoxyphene hcl, amitriptyline hcl,

carisoprodol/codeine phos/asa, morphine sulfate, multivitamins, naproxen sodium, orphenadrine citrate, temazepam.

Preferred examples of therapeutic agents for SLE (Lupus) in which an antibody or an antigen binding portion can be combined include the following: NSAIDS, for example, diclofenac, naproxen, ibuprofen, piroxicam, indomethacin; COX2 inhibitors, for example, Celecoxib, rofecoxib, valdecoxib; anti-malarials, for example, hydroxychloroquine; Steroids, for example, prednisone, prednisolone, budenoside, dexamethasone; Cytotoxics, for example, azathioprine, cyclophosphamide, mycophenolate mofetil, methotrexate; inhibitors of PDE4 or purine synthesis inhibitor, for example Cellcept. Antibodies of the invention or antigen binding portions thereof, may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid, olsalazine, Imuran and agents which interfere with synthesis, production or action of proinflammatory cytokines such as IL-1, for example, caspase inhibitors like IL-1β converting enzyme inhibitors and IL-1ra. Antibodies of the invention or antigen binding portion thereof may also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors; or molecules that target T cell activation molecules, for example, CTLA-4-IgG or anti-B7 family antibodies, anti-PD-1 family antibodies. Antibodies of the invention, or antigen binding portions thereof, can be combined with IL-11 or anti-cytokine antibodies, for example, fonotolizumab (anti-IFNg antibody), or anti-receptor receptor antibodies, for example, anti-IL-6 receptor antibody and antibodies to B-cell surface molecules. Antibodies of the invention or antigen binding portion thereof may also be used with LJP 394 (abetimus), agents that deplete or inactivate B-cells, for example, Rituximab (anti-CD20 antibody), lymphostat-B (anti-BlyS antibody), TNF antagonists, for example, anti-TNF antibodies, D2E7 (PCT Publication No. WO 97/29131; HUMIRA), CA2 (REMICADE), CDP 571, TNFR-Ig constructs, (p75TNFRIgG (ENBREL) and p55TNFRIgG (LENERCEPT)).

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the

desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.1-20 mg/kg, more preferably 1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

II. IL-18 responsive genes

IL-18 is expressed in macrophages, dendritic cells, Kupffer cells, microglia, epithelial cells, keratinocytes, intestinal epithelial cells, chondrocytes, synovial fibroblasts and osteoblasts, as well as within the adrenal cortex and pituitary gland. In some cells, such as human monocytes and dendritic cells, expression is constitutive, whereas in other cells it must be induced de novo.

Apart from interferon gamma expression, little is known about other genes induced by IL-18 alone or in concert with other cytokines.

One embodiment of the invention provides a method for regulating gene expression of a gene of interest comprising the steps of providing IL-18 or an IL-18 modulator; and contacting IL-18 or

the modulator to a cell wherein the gene of interest is selected from the group consisting of the genes presented in the following table.

Table 3 IL-18 Responsive Genes

Genbank ID	Gene Name	Unigene Comment
NM_000389	p21	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
NM_002198	IRF1	interferon regulatory factor 1
NM_002163	ICSBP1	interferon consensus sequence binding protein 1
NM_006144	GZMA	granzyme A
NM_006515	SETMAR	SET domain and mariner transposase fusion gene
NM_007185	TNRC4	trinucleotide repeat containing 4
NM_002288	LAIR2	leukocyte-associated Ig-like receptor 2
NM_003661	APOL1	apolipoprotein L, 1
NM_021958	HLX1	H2.0-like homeo box 1 (Drosophila)
NM_001335	CTSW	cathepsin W (lymphopain)
Hs.382006	FCGR1B	FcRI b form (AA 1-344)
NM_020125	BLAME	leucine aminopeptidase 3
NM_007210	GALNT6	UDP-N-acetyl-alpha-D-galactosamine
NM_021798	IL21R	interleukin 21 receptor
NM_013324	CISH	cytokine inducible SH2-containing protein
M11313	A2M	alpha-2-macroglobulin
D88152	ACATN	acetyl-Coenzyme A transporter
NM_001103	ACTN2	actinin, alpha 2
U37519	ALDH8	aldehyde dehydrogenase 8
NM_000697	ALOX12	arachidonate 12-lipoxygenase
J03600	ALOX5	arachidonate 5-lipoxygenase
NM_014578	ARHD	ras homolog gene family, member
S66793	ARR3	arrestin 3, retinal (X-arrestin)
U47054	ART3	ADP-ribosyltransferase 3
L19871	ATF3	activating transcription factor 3
M81181	ATP1B2	ATPase, Na+/K+ transporting
NM_001188	BAK1	BCL2-antagonist/killer 1
U15460	BATF	basic leucine zipper transcription factor, ATF-like
NM_014417	BBC3	Bcl-2 binding component 3
Z23115	BCL2L1	BCL2-like 1
NM_001713	BHMT	betaine-homocysteine methyltransferase
U45878	BIRC3	baculoviral IAP repeat-containing 3
U37546	BIRC3	baculoviral IAP repeat-containing 3
U72649	BTG2	BTG family, member 2
U49187	C6ORF32	chromosome 6 open reading frame 32
J03507	C7	complement component 7
U50360	CAMK2G	CaM kinase II gamma
XM_071866	CAT56	CAT56 protein
NM_005623	CCL8	
Z32765	CD36	CD36 antigen (collagen type I/ TSP receptor)
HG2981-HT3127	CD44	CD44 antigen

Z11697	CD83	CD83 antigen
XM_071866	CDR2	cerebellar degeneration-related protein (62kD)
U51096	CDX2	caudal type homeo box transcription factor 2
M83667	CEBPD	CCAAT/enhancer binding protein (C/EBP), delta
D87469	CELSR2	cadherin, EGF LAG seven-pass G-type receptor 2
L07765	CES1	carboxylesterase 1
U66468	CGR11	cell growth regulatory with EF-hand domain
X14830	CHRNB1	cholinergic receptor, nicotinic, beta polypeptide 1
L29217	CLK3	CDC-like kinase 3
X15880	COL6A1	collagen, type VI, alpha 1
NM_001851	COL9A1	collagen, type IX, alpha 1
M27691	CREB1	cAMP responsive element binding protein 1
M37435	CSF1	colony stimulating factor 1 (macrophage)
HG3548-HT3749	CUTL1	cut (CCAAT displacement protein)
X13589	CYP19	cytochrome P450, subfamily XIX
X16866	CYP2D7AP	cytochrome P450, subfamily IID
X59131	D13S106E	highly charged protein
NM_004393	DAG1	dystroglycan 1
U73328	DLX4	distal-less homeobox 4
L19267	DMWD	dystrophia myotonica, WD repeat motif
U53445	DOC1	downregulated in ovarian cancer 1
X68277	DUSP1	dual specificity phosphatase 1
U48807	DUSP4	dual specificity phosphatase 4
NM_001950	E2F4	E2F transcription factor 4, p107/p130-binding
U87269	E4F1	E4F transcription factor 1
M57730	EFNA1	ephrin-A1
X52541	EGR1	early growth response 1
J04076	EGR2	early growth response 2 (Krox-20 homolog)
X63741	EGR3	early growth response 3
L07077	EHHADH	enoyl-Coenzyme A
M62831	ETR101	immediate early protein
M60830	EVI2B	ecotropic viral integration site 2B
U53786	EVPL	envoplakin
NM_001988	EVPL	envoplakin
NM_000141	FCGBP	Fc fragment of IgG binding protein
M23668	FDX1	ferredoxin 1
U60062	FEZ1	fasciculation & elongation protein zeta 1 (zygin I)
NM_000141	FGFR2	fibroblast growth factor receptor 2
U49973	FLJ10803	hypothetical protein FLJ10803
U89995	FOXE1	forkhead box E1 (thyroid transcription factor 2)
U27326	FUT3	fucosyltransferase 3
A28102	GABRA3	gamma-aminobutyric acid (GABA) receptor
M25667	GAP43	growth associated protein 43
L34357	GATA4	GATA-binding protein 4
U19523	GCH1	GTP cyclohydrolase 1
L01406	GHRHR	growth hormone releasing hormone receptor

U03486	GJA5	gap junction protein, alpha 5, 40kD (connexin 40)
X68285	GK	glycerol kinase
Z18859	GNAT2	guanine nucleotide binding protein (G protein)
HG870-HT870	GOLGA3	golgi autoantigen, golgin subfamily a, 3
D49958	GPM6A	glycoprotein M6A
D43772	GRB7	growth factor receptor-bound protein 7
AC000099	GRM8	glutamate receptor, metabotropic 8
M57731	GRO2	GRO2 oncogene
X53800	GRO3	GRO3 oncogene
M91036	HBG2	hemoglobin, gamma G
D16583	HDC	histidine decarboxylase
X64877	HFL3	H factor (complement)-like 3
X58431	HOXB6	homeo box B6
M16937	HOXB7	homeo box B7
NM_014468	HPX42B	haemopoietic progenitor homeobox
X92814	HREV107	similar to rat HREV107
L19314	HRY	hairy (Drosophila)-homolog
M26665	HTN3	histatin 3
D10995	HTR1B	5-hydroxytryptamine (serotonin) receptor 1B
L41147	HTR6	5-hydroxytryptamine (serotonin) receptor 6
M24283	ICAM1	intercellular adhesion molecule 1 (CD54)
S81914	IER3	immediate early response 3
J03171	IFNAR1	interferon (alpha, beta and omega) receptor 1
J00219	IFNG	interferon, gamma
NM_000619	IFNG	interferon, gamma
NM_000585	IL15	interleukin 15
U31628	IL15RA	interleukin 15 receptor, alpha
X04500	IL1B	interleukin 1, beta
M27492	IL1R1	interleukin 1 receptor, type I
X01057	IL2RA	interleukin 2 receptor, alpha
M26062	IL2RB	interleukin 2 receptor, beta
Y00081	IL6	interleukin 6 (interferon, beta 2)
Y00787	IL8	interleukin 8
Z31695	INPP5A	inositol polyphosphate-5-phosphatase, 40kD
X06256	ITGA5	integrin, alpha 5
X57206	ITPKB	inositol 1,4,5-trisphosphate 3-kinase B
U20734	JUNB	jun B proto-oncogene
NM_014879	KIAA0001	putative G protein coupled receptor for UDP-glucose
D31762	KIAA0057	TRAM-like protein
D42038	KIAA0087	KIAA0087 gene product
NM_005551	KIAA0133	KIAA0133 gene product
NM_014846	KIAA0196	KIAA0196 gene product
X06182	KIT	v-kit oncogene homolog
NM_005551	KLK2	kallikrein 2, prostatic
X07730	KLK3	kallikrein 3, (prostate specific antigen)
M13955	KRT7	keratin 7
M57710	LGALS3	lectin, galactoside-binding, soluble, 3 (galectin 3)

S83362	LIFR	leukemia inhibitory factor receptor
NM_002314	LIMK1	LIM domain kinase 1
NM_005569	LIMK2	LIM domain kinase 2
U49957	LPP	LIM domain-containing
U89922	LTB	lymphotoxin beta (TNF superfamily, member 3)
X14008	LYZ	lysozyme (renal amyloidosis)
U59914	MADH6	MAD) homolog 6
D14497	MAP3K8	mitogen-activated protein kinase kinase kinase 8
X59727	MAPK4	mitogen-activated protein kinase 4
NM_000429	MAT1A	methionine adenosyltransferase I, alpha
HG1877-HT1917	MBP	myelin basic protein
HG3115-HT3291	MBP	myelin basic protein
U43944	ME1	malic enzyme 1, NADP(+)-dependent, cytosolic
X72755	MIG	monokine induced by gamma interferon
NM_021230	MLL3	myeloid/lymphoid or mixed-lineage leukemia3
NM_005951	MT1H	metallothionein 1H
X78710	MTF1	metal-regulatory transcription factor 1
X70991	NAB2	NGFI-A binding protein 2 (ERG1 bp 2)
M32011	NCF2	neutrophil cytosolic factor 2
S77763	NFE2	nuclear factor (erythroid-derived 2), 45kD
M58603	NFKB1	nuclear factor kappa B (p105)
S76638	NFKB2	nuclear factor kappa B
M69043	NFKBIA	nuclear factor kappa B
U91616	NFKBIE	nuclear factor kappa B
D86425	NID2	nidogen 2
L13740	NR4A1	nuclear receptor subfamily 4, group A, member 1
U44848	NRF1	nuclear respiratory factor 1
U79251	OPCML	opioid-binding protein/cell adhesion molecule-like
HG4115-HT4385	OR1E3P	olfactory receptor
M27288	OSM	oncostatin M
AF000234	P2RX4	purinergic receptor P2X
D50640	PDE3B	phosphodiesterase 3B, cGMP-inhibited
L20971	PDE4B	phosphodiesterase 4B, cAMP-specific
L10343	PI3	protease inhibitor 3, skin-derived (SKALP)
U77735	PIM2	pim-2 oncogene
NM_003579	PIP5K2A	phosphatidylinositol-4-phosphate 5-kinase
U17034	PLA2R1	phospholipase A2 receptor 1, 180kD
AB000584	PLAB	prostate differentiation factor
X63131	PML	promyelocytic leukemia
D11428	PMP22	peripheral myelin protein 22
NM_032940	POLR2C	polymerase (RNA) II polypeptide
NM_005035	POLRMT	polymerase (RNA) mitochondrial (DNA directed)
NM_003579	POU2F2	POU domain, class 2, transcription factor 2
M18255	PRKCB1	protein kinase C, beta 1

L01087	PRKCQ	protein kinase C, theta
D38128	PTGIR	prostaglandin I2 (prostacyclin) receptor (IP)
Y10375	PTPNS1	tyrosine phosphatase, non-receptor substrate 1
D15049	PTPRH	protein tyrosine phosphatase, receptor type, H
M31166	PTX3	pentaxin-related gene,
U59877	RAB31	RAB31, member RAS oncogene family
NM_003579	RAD54L	RAD54 (S.cerevisiae)-like
U64675	RANBP2L1	RAN binding protein 2-like 1
S57153	RBBP1	retinoblastoma-binding protein 1
NM_002903	RCV1	recoverin
NG_000013	RDBP	RD RNA-binding protein
X75042	REL	v-rel
M83221	RELB	v-rel
NM_000537	REN	renin
U22314	REST	RE1-silencing transcription factor
S59049	RGS1	regulator of G-protein signalling 1
U70426	RGS16	regulator of G-protein signalling 16
U22377	RLF	rearranged L-myc fusion sequence
U38480	RXRG	retinoid X receptor, gamma
L10338	SCN1B	sodium channel polypeptide
M23178	SCYA3	small inducible cytokine A3
M69203	SCYA4	small inducible cytokine A4
NM_005409	SCYB11	small inducible cytokine subfamily B: CXC11
D79206	SDC4	syndecan 4 (amphiglycan, ryudocan)
NM_005065	SEL1L	sel-1 (suppressor of lin-12, C.elegans)-like
NM_004186	SEMA3F	semaphorin 3F
J03764	SERPINE1	nexin, plasminogen activator inhibitor type 1
NM_006802	SF3A3	splicing factor 3a, subunit 3, 60kD
HG3925-HT4195	SFTPA2	surfactant, pulmonary-associated protein A2
D89077	SLA	Src-like-adapter
NM_003037	SLAM	signaling lymphocytic activation molecule
M91463	SLC2A4	solute carrier family 2 glucose transporter
D82326	SLC3A1	solute carrier family 3
L05568	SLC6A4	solute carrier family 6 (serotonin),
U96094	SLN	sarcolipin
X83301	SMA3	SMA3
D21267	SNAP25	synaptosomal-associated protein, 25kD
L31529	SNTB1	syntrophin, dystrophin-associated protein A1,
HG961-HT961	SOS1	son of sevenless (Drosophila) homolog 1
M62800	SSA1	(52kD, ribonucleoprotein autoantigen SS-A/Ro)
NM_021014	SSX3	synovial sarcoma, X breakpoint 3
Z35093	SURF1	surfeit 1
NM_005816	TACTILE	T cell activation, increased late expression
L25444	TAF2E	TATA box binding protein (TBP)-associated factor
M95787	TAGLN	transgelin
NM_005421	TAL2	T-cell acute lymphocytic leukemia 2

L47345	TCEB3	transcription elongation factor B (110kD, elongin A)
M57732	TCF1	hepatic nuclear factor (HNF1)
NM_003205	TCF12	helix-loop-helix transcription factors 4
M96956	TDGF1	teratocarcinoma-derived growth factor 1
U19878	TMEFF1	transmembrane with EGF and follistatin like
M92357	TNFAIP2	tumor necrosis factor, alpha-induced protein 2
M59465	TNFAIP3	tumor necrosis factor, alpha-induced protein 3
X83490	TNFRSF6	tumor necrosis factor receptor member 6
U37518	TNFSF10	tumor necrosis factor member 10
NM_003294	TPSB1	tryptase beta 1
U19261	TRAF1	TNF receptor-associated factor 1
U78798	TRAF6	TNF receptor-associated factor 6
S69790	WASF3	WAS protein family, member 3
U53476	WNT7A	wingless-type MMTV integration site family
L15309	ZNF141	zinc finger protein 141 (clone pHZ-44)
U78722	ZNF165	zinc finger protein 165
HG4333-HT4603	ZNF79	zinc finger protein 79 (pT7)
X57809		lambda light chain variable region
HG3111-HT3287		Homo sapiens clone HH409 unknown
U79249		Human clone 23839 sequence
AB000464		clone:RES4-24A
HG4593-HT4998		voltage-gated sodium channel (SCN1A)
X77744		Homo sapiens for FLJ00032 protein, partial
U79248		Human clone 23826 sequence
AI420129		ESTs

Method of identifying genes regulated by the IL-18 are disclosed in Example 3. These studies showed that IL-18 is a bona fide proinflammatory cytokine and can directly regulate the expression of several genes encoding other proinflammatory mediators. Studies using human blood samples show that many responses to IL-18 occur widely in the human population and demonstrate their utility as biochemical markers of IL-18, and consequently anti-IL18, function.

Modulators of IL-18 can be agonists and antagonist. Preferably the modulator is a binding protein or a neutralizing binding protein.

Exemplary IL-18 inhibitors include, but are not limited to, antibodies, and fragments thereof, that bind to IL-18; antibodies that bind to IL-18R; antibodies that bind to IL-18RAcP; IL-18bp; IL-18R fragments (e.g., a solubilized extracellular domain of the IL-18 receptor); peptides that bind to IL-18 and reduce or prevent its interaction with IL-18R; peptides that bind to IL-18R and reduce or prevent its interaction with IL-18 or with IL-18RAcP; peptides that bind to IL-18RAcP and reduce or

prevent its interaction with IL-18R; and small molecules that reduce or prevent IL-18 production or the interaction between any of IL-18, IL-18R, and IL-18RAcP.

Certain IL-18 inhibitors are described, e.g., in US Pat. No. 5,912,324, issued July 14, 1994; EP 0 962 531, published Dec. 8, 1999; EP 712 931, published Nov. 15, 1994; US Pat. No. 5,914,253, issued July 14,1994; WO 97/24441, published July 10, 1997; US Pat. No. 6,060,283, issued May 9, 2000; EP 850 952, published Dec. 26, 1996; EP 864 585, published Sep. 16, 1998; WO 98/41232, published Sep. 24, 1998; US Pat. No. 6,054,487, issued April 25, 2000; WO 99/09063, published Aug 14,1997; WO 99/22760, published Nov. 3, 1997; WO 99/37772, published Jan. 23,1998; WO 99/37773, published March 20, 1998; EP 0 974 600, published Jan. 26, 2000; WO 00112555, published Mar.9, 2000; Japanese patent application JP 111,399194, published Oct. 31, 1997; Israel patent application IL 121554 A0, published Feb. 8, 1998; which are incorporated herein by reference for any purpose.

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the invention described herein are obvious and may be made using suitable equivalents without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

Examples

Example 1: Production and Characterization of recombinant IL-18

Example 1.1: **Assays to determine biological activity of IL-18**

Throughout Example 1 and Example 2 the following assays were used to determine biological activity of IL-18 unless otherwise stated.

Example 1.1.A: **KG-1 Bioassay**

KG-1 (ATCC #CCL-246) is a human myelomonocytic cell line that constitutively expresses low levels of functional IL-18 receptor. Treatment with TNF up-regulates both the IL-18Rα and β subunits of the functional IL-18 receptor on these cells. The KG-1 bioassay was performed by incubating TNF-treated KG-1 cells with recombinant human IL-18 (rhu-IL-18) and determining the level of IL-18-induced human IFNγ production by an ELISA. (Konishi, K., et al (1997) *J. Immunol. Methods* 209:187-191). The KG-1 bioassay was used to determine the neutralization potency of IL-

18 antagonists. For example, anti-IL-18 antibodies were incubated with different concentrations of rhu-IL-18 and then incubated with TNF-treated KG-1 cells in a 96-well plate for 16-18 hours at 37°C. The supernatants collected and assayed for human IFNγ levels by an ELISA. This assay can measure IC50 values down to $4x10^{-11}$ - $6x10^{-11}$ M of an IL-18 antagonist.

Example 1.1.B: **Human Whole Blood Assay**

Briefly, the Human Whole Blood Assay (WBA) determines neutralization potency of IL-18 antagonists against natural IL-18 within a physiological context. In this assay, the readout was inhibition of endogenous IL-18-dependent human IFNγ production. Whole blood was stimulated with LPS (1 μg/mL) plus IL-12 (50 pg/mL) in the presence or absence of IL-18 antagonists at 37°C. Human IFNγ concentrations were determined by ELISA 18-24 hrs post-LPS plus IL-12 stimulation.

Example 1.1.C: **Receptor Binding Assay**

Briefly, in the Receptor Binding Assay (RBA), ^{125}I labeled rhu-IL-18 was used to determine binding of IL-18 to IL-18 receptor. ^{125}I-rhu-IL-18 binds specifically to the IL-18Rαβ on TNF-treated KG-1 cells (~7,000 sites/cell). ^{125}I-rhu-IL-18 has the same specific activity as unlabeled IL-18 and can be competed off by unlabeled IL-18.

Two modes of inhibition, A and B, were defined. In neutralization Mode A , binding of IL-18 to the high affinity IL-18 receptor (IL-18Rαβ) was not effected, but IL-18-mediated signal transduction (i.e. IFNγ production) was blocked. In neutralization Mode B binding of IL-18 to IL-18Rαβ was blocked and thereby no subsequent receptor-mediated signaling occured.

Example 1.2: **Production of recombinant IL-18**

Example 1.2.A: **Plasmid construction, Expression and purification of human proIL-18**

Recombinant human IL-18 was generated by expressing the precursor form of IL-18 in SF-9 insect cells. Using standard molecular biological methods well known in the art, full-length human pro-IL-18 cDNA was generated using specific PCR primers based on published sequence (Ushio, S., et al. (1996) J. Immunol. 156:4274-4279) and subsequently cloned into the baculovirus (BV) transfer vector pVL1393. (BD Biosciences, San Jose, CA; Cat# 51-21201P) The 5' PCR primer used to generate full-length human pro-IL-18 cDNA contained sequences encoding a 6-Histidine region such

that the N-terminus of the proIL-18 contained a 6-HIS-tag. SF9 insect cells were infected with baculovirus harboring pVL1393 vector containing IL-18 cDNA. Infected SF9 cells were lysed and the lysates were run over a nickel column to purify recombinant HIS-tagged proIL-18 (rhu pro IL-18). (BD Biosciences, San Jose, CA; Cat# 554802) The recombinant HIS-tagged proIL-18 was processed further by digesting with human caspase-1 to generate biologically active IL-18 (mature IL-18). (Ghayur T., (1997) *Nature* 386:619-623).

Example 1.2.B: NEM treatment of IL-18

Recombinant human IL-18 obtained from Hayashibara Biochemical Laboratories, Japan, displayed batch variation in specific activity and in IL-18 binding affinity. IL-18 contained disulphide bonds between various pairs of the four cysteines in mature IL-18. These caused structural and functional heterogeneity, and variations between batches. Homology modeling of human IL-18 using IL-1b coordinates showed that cysteine residues at positions 38 and 68 of mature human IL-18 are exposed and therefore reactive.

Recombinant human IL-18 from Example 1.2.A was treated with N-ethyl maleiamide (NEM) to protect the cysteines from oxidation. NEM-IL-18 was monomeric, did not form aggregates, was stable, and retained high specific activity over time. NEM-IL-18 retained neutralizing epitopes because anti-huIL-18 neutralizing antibodies bound and neutralized NEM-IL-18. Despite NEM treatment of IL-18, neutralizing epitopes on NEM-IL-18 were preserved as determined by the ability of anti-IL-18 antibodies to neutralize biological activity of both NEM-IL-18 and natural human IL-18 in human WBA. NEM-IL-18 was used for assay optimization and selection and initial characterization of fully human anti-human -IL-18 mAbs.

Example 1.2.C: Generation and characterization of 4C/A mutant of IL-18

The 4C/A mutant of IL-18 was generated by mutating the four Cysteine residues in mature IL-18 to Alanine ("4C/A-huL-18"). Comparison of 4C/A mutant of IL-18 with NEM-huIL-18, as summarized in Table 4 below, showed that the two proteins were indistinguishable in biological and biochemical properties. Both 4C/A mutant of IL-18 and NEM-huIL-18 were monomeric by dynamic light scattering (DLS) and size exclusion chromatography (SEC) analysis, and similar in conformation and physical stability by circular dichroism analysis. The biological activity of 4C/A mutant of IL-18 and NEM-huIL-18 were the same in the KG-1 assay, and both forms of IL-18 bound IL-18BP and anti-IL-18 antibodies with similar affinity. 4C/A-huL-18 was not subject to oxidative instability and was readily expressed at high levels in *E. coli*.

Table 4 **Comparison of NEM-IL-18 and 4C/A-huIL-18 shows that they are equivalent in measurements of conformational and oligomeric purity, physical stability, and binding to antibodies or cell-bound receptors.**

Properties	Measurements	NEM-huIL-18	(4C/A)-huIL-18
Oligomeric state	SEC	Monomeric	Monomeric
	DLS	Monomeric	Monomeric
Confirmation	CD (wavelength scan)	CD minimum at 210 nm	CD minimum at 210 nm
Stability	CD (temperature scan)	Stable up to 40° C	Stable up to 40° C
Bioactivity	IFNγ production by 2 ng/mL IL-18	8 ng/mL IFNγ	8 ng/mL IFNγ
Epitopes	Neutralization of IFNγ production by reference binder	Neutralized by IL-18BP-Fc, 125-2H and IL-18Rα	Neutralized by IL-18BP-Fc, 125-2H and IL-18Rα
	Biacore (K_D)	IL-18BP-Fc: 0.098 nM 125-2H: 0.2 nM 2.5(E)mg1: 0.3 nM	IL-18BP-Fc: 0.135 nM 125-2H: 0.2 nM 2.5(E)mg1: 0.2 nM

Example 1.2.D: **<u>Generation and characterization of Biotinylated rhuIL-18 (biot-IL-18)</u>**

Biotinylation of NEM-IL-18 from Example 1.2.B was performed on lysine residues using standard techniques well known in the art, (Sulfo-NHS-LC-Biotin, Pierce, Rockford, IL; Cat#21335), and the biotinylated rhu-IL-18 (biot-IL-18) obtained was a heterogeneous mixture containing species with 1, 2, 3, or 4 biotins per huIL-18. Furthermore, species with 2 or 3 biotins per rhuIL-18 were the major species in biot-IL-18. Biot-IL-18 was biologically active, bound anti-IL-18 antibodies as determined by ELISA, and was neutralized by all neutralizing anti-huIL-18 antibodies tested. Biot-IL-18 bound KG-1 cells expressing IL-18Rαβ on their surface with high affinity, and the biot-IL-18 on the surface of KG-1 cells was detected by FACS analysis using anti-biotin antibodies (Sigma-

Aldrich, St Louis, MO; cat# B 3640). Thus biotinylation does not interfere with receptor binding and does not mask neutralizing epitopes of rhuIL-18.

Example 1.2.E: Generation and characterization of ^{125}I Labeled rhuIL-18

The lysine residues on NEM-IL-18 from Example1.2.B were labeled with ^{125}I using conditions specified by Amersham (Piscataway, NJ; Cat # IM5861). ^{125} I-labeled IL-18 retained its specific activity, was competed by non-modified IL-18, and bound specifically to IL-18R on KG-1 cells. Binding of ^{125} I-labeled IL-18 to IL-18 receptor was blocked by neutralizing anti-huIL-18 monoclonal antibodies. Thus iodination did not affect receptor binding of IL-18 and did not mask neutralizing epitopes on the IL-18. ^{125}I-labeled IL-18 was used to determine the neutralization mode and potency of anti-IL-18 antibodies in the Receptor Binding Assay.

EXAMPLE 2: Generation and isolation of anti IL-18 antibodies

Example 2.1: Assays to identify anti-IL-18 antibodies

Throughout Example 3 the following assays were used to identify and characterize anti-IL-18 antibodies unless otherwise stated.

Example 2.1.A: ELISA

An ELISA was developed to screen for antibodies that bind human IL-18. In this ELISA, biotinylated NEM-huIL-18 (see Example 1.2.B) was captured by either goat anti-biotinylated IgG or on to streptavidin coated plates. Hybridoma or B cell supernatants were applied and IL-18-bound antibodies were detected using HRP-conjugated anti-human IgGs, following standard ELISA protocols well known in the art.

Example 2.1.B: Affinity Determinations using BIACORE technology

The BIACORE assay (Biacore, Inc, Piscataway, NJ) determines the affinity of antibodies with kinetic measurements of on-, off-rate constants. Antibodies are captured on a biosensor chip by means of a covalently linked secondary antibody (e.g. goat anti-human IgG or anti-mouse IgG) and then varying concentrations of recombinant IL-18 are applied. Binding is recorded as a function of time and kinetic rate constants are calculated. In this assay, on-rates as fast as $10^6 M^{-1}s^{-1}$ and off-rates as slow as 10^{-6} s^{-1} can be measured.

Example 2.1.C: Epitope Mapping

BIACORE technology was used for mapping the epitopes recognized by IL-18 antagonists such as anti-IL-18 antibodies. Briefly, one IL-18 antagonist was captured on the Biacore chip and rhuIL-18 was bound to the immobilized reagent. Binding of another anti-IL-18 antagonist to this complex was then tested. Simultaneous binding of two reagents demonstrates that the two recognize distinct epitopes.

Example 2.2: Generation of Anti-IL-18 HuMAbs Using XENOMOUSE

The XENOMOUSE transgenic mouse technology (Abgenix, Inc., Fremont, CA) was employed to obtain fully human anti-human IL-18 monoclonal antibodies (HuMAbs). This technology consists of transgenic mice carrying human variable heavy chain locus carrying VH, DH, and JH, Cmu, Cdelta and a single human IgG constant heavy chain locus and light chain gene loci. Upon immunization with an antigen of interest, these mice generate fully human antibodies to the antigen.

Example 2.2.A: Immunization of XENOMOUSE with IL-18 antigen

XENOMOUSE animals were immunized via footpad route for all injections. Total volume of each injection was 50ul per mouse, 25ul per footpad. Initial immunization injection contained 40ug human IL-18 (NEM-rhuIL-18) in pyrogen-free DPBS admixed 1:1 v/v with TiterMax Gold per mouse. Subsequent boosts were made with 40ug Human IL-18 in pyrogen free DPBS admixed with 25ug of Adju-Phos (aluminum phosphate gel) per mouse for six times, then a final boost of 40ug Human IL-18 in pyrogen free DPBS without adjuvant per mouse. The animals were immunized on days 0, 4, 8, 11, 17, 21, 25 and 35 for this protocol. Fusions were performed on day 39. Following the immunization regimen described above, mice were euthanized, then inguinal and Lumbar lymph nodes were recovered.

Example 2.2.B: Generation of Hybridoma

Lymphocytes were released by mechanical disruption of the inguinal and Lumbar lymph nodes, obtained according to Example 2.2.A, using a tissue grinder, and depleted of T cells by CD90 negative selection. Hybridoma fusion was performed by mixing washed enriched B cells and non-secretory myeloma P3X63Ag8.653 cells purchased from ATCC, cat. # CRL 1580 (Kearney et al, J. Immunol. 123, 1979, 1548-1550) at a ratio of 1:1. The cell mixture was gently pelleted by

centrifugation at 800 g. After complete removal of the supernatant, the cells were treated with 2-4 mL of Pronase solution (CalBiochem, San Diego, CA; cat. # 53702; 0.5 mg/ml in PBS) for no more than 2 minutes. Then 3–5 ml of FBS was added to stop the enzyme activity and the suspension was adjusted to 40 ml total volume using electro cell fusion solution, ECFS (0.3M Sucrose, Sigma-Aldrich, St Louis, MO; Cat# S7903, 0.1mM Magnesium Acetate, Sigma, Cat# M2545, 0.1mM Calcium Acetate, Sigma-Aldrich, St Louis, MO; Cat# C4705). The supernatant was removed after centrifugation and the cells were resuspended in 40 ml ECFS. This wash step was repeated and the cells again were resuspended in ECFS to a concentration of $2x10^6$ cells/ml. Electro-cell fusion was performed using a fusion generator, model ECM2001, Genetronic, Inc., San Diego, CA. The fusion chamber size used was 2.0 ml using the following instrument settings: Alignment condition: voltage: 50 v, time: 50 s; Membrane breaking at: voltage: 3000 v, time: 30 μs; Post-fusion holding time: 3 s.

After fusion, the cells were resuspended in hybridoma fusion medium: DMEM (JRH Biosciences), 15 %FBS (Hyclone), containing 0.5XHA (Sigma-Aldrich, St Louis, MO; cat. # A9666), and supplemented with L-glutamine, pen/strep, OPI (oxaloacetate, pyruvate, bovine insulin) (all from Sigma) and IL-6 (Boehringer Mannheim, Indianapolis, IN) for culture at 37 °C and 10% CO^2 in air. Cells were plated in flat bottomed 96-well tissue culture plates at $4x10^4$ cells per well. Cultures were maintained in hybridoma fusion medium for 2 weeks before transfer to Hybridoma medium: DMEM (JRH Biosciences, Lenexa, KS), 15 %FBS (Hyclone, Logan, Utah), and supplemented with L-glutamine, pen/strep, OPI (oxaloacetate, pyruvate, bovine insulin) (all from Sigma) and IL-6 (Boehringer Mannheim, Indianapolis, IN). Hybridomas were selected for by survival in 0.5XHA hybridoma fusion medium and supernatants from those wells containing hybridomas were screened for antigen reactivity by ELISA. The ELISA format entailed incubating supernatants on antigen coated plates (human IL-18 coated plates) and detecting human anti-human IL-18 binding antibodies using horseradish peroxidase (HRP) labeled mouse anti-human IgG, then the all positive samples were confirmed by two sets of ELISA in parallel, which entailed incubating supernatants on antigen coated plates (human IL-18 coated plates) and detecting human anti-Human IL-18 binding antibodies using horseradish peroxidase (HRP) labeled mouse anti-human Gamma and Kappa chain.

Cloning was performed on selected antigen-positive wells using limited dilution plating. Plates were visually inspected for the presence of single colony growth and supernatants from single colony wells then screened by antigen-specific ELISAs as described above. Highly reactive clones

were assayed to verify purity of human gamma and kappa chain by multiplex ELISA using a Luminex instrument.

Example 2.2.C: XENOMAX Technology

Alternatively, lymphocytes obtained in Example 2.2.B were subjected to Selective Lymphocyte Antibody-generation Method (SLAM), which defines XENOMAX antibody selection technology (Abgenix, Inc., Fremont, CA). Single B cells were plated in 96 well plates B cells producing human monoclonal antibodies to desired antigen (human IL-18) were identified by a plaque forming cell assay (Babcook, J.S., Leslie, K.B., Olsen, O.A., Salmon, R.A., and Schrader, J.W. Isolation of functional antibody genes from single lymphocytes of defined antigen-specificity. *Proc. Natl. Acad. Sci. USA*, 93:7843-7848, 1996) and IgG genes were cloned by single-cell RT-PCR of isolated B cells using 5' primers for VH and Vk leader sequences and 3' primers specific for human Cgamma and Ckappa. The recombinant IgG genes obtained were expressed in mammalian cells as described in Examples 2.2.E and 2.2.G.

Example 2.2.D: Identification of anti-IL-18 antibodies

Hybridomas and B-cell producing antibodies that bound IL-18, generated according to Examples 2.2.B and 2.2.C, were identified using biotinylated IL-18 ELISA (see Example 2.1.A). Hybridomas and B-cell supernatants containing antibodies that bound IL-18 were then tested for IL-18 neutralization potency in the KG-1 bioassay performed according to Example 1.1.A. Neutralizing anti-IL-18 antibodies (from hybridoma and SLAM aproaches) were subcloned into a mammalian expression vector, expressed in COS cells, purified and re-tested in the KG-1 bioassay (seeTable 5).

Table 5 Neutralization Potency of Anti-IL-18 HuMAbs in KG-1 Bioassay

HuMAb#	KG-1 Assays (IC_{50},M)
	NEM-rhuIL-18
Hybridoma	
2.5.1	3E-10; 4E-10
2.13.1	2E-10; 1E-10; 7E-11
2.3.3	1E-9; 2E-10; 7E-10
XENOMAX	

215	1E-10; 3E-10; 1E-10;
444	1E-10; 2E-10; 2E-10
478	7E-10; 2E-9; 3E-10
435	8E-10; 7E-10; 4E-10;
413	1E-9; 7E-10; 7E-10
581	7E-10; 3E-10; 3E-9
231	1E-10; 3E-11; 2E-9
521	6E-10; 3E-10; 2E-9
336	7E-10
351	2E-10
490	5E-10
550	TBD
268	7E-9

The variable regions of the antibodies in Table 5 are described inTable1

Example 2.2.E: Subloning of neutralizing anti-IL-18 HuMAbs into mammalian expression vector

Genes for the heavy and light chains of antibodies were cloned in pCDNA (Invitrogen, Carlsbad, Ca) vectors under control of the CMV promoter following manufacturer's instruction. These plasmids with human genomic Gamma-2 and kappa sequences were used to cotransfect COS cells by electroporation with heavy and light chains corresponding to each clone employing standard conditions well known in the art.

Cells were allowed to recover, grow, and secrete antibodies for 72 hours in serum free DMEM supplemented with glutamine. Culture supernatants were then collected, clarified by centrifugation and filtration, and put over Protein-A resin. Columns were washed with PBS, antibodies eluted with low pH buffer, and quickly neutralized with 1M Tris solution. Antibody preps were buffer-exchanged with PBS on Amicon-30 spin filters. Concentration and purity of antibodies were analyzed by spectrometry at OD 280 and SDS-PAGE before they were tested for IL-18 neutralization potency.

To achieve greater expression levels of human antibodies in COS cells, the heavy and light chains of some antibodies were subcloned into vector pEF-BOS (Mizushima, S. and Nagata, S. (1990) Nucleic acids Research Vol 18, No. 17) under control of elongation factor promoter.

In short, PCR primers for heavy chain variable regions were designed in such a way that they could be inserted into a cassette pEF-BOS plasmid containing an IgG signal peptide and the sequence of human IgG1 constant region [wild type (SEQ ID No. 2) or inactive mutant (SEQ ID No. 3)]. The forward V_H PCR primer contained restriction site NruI, as did the nucleotide sequence of the signal peptide. The reverse V_H PCR primer contained SalI restriction site that was also engineered into the 5' end of the gamma-1 Fc sequence. The V_H PCR fragments were digested with NruI/SalI and cloned into the pEF-BOS human IgG1 wild type or pEF-BOS human IgG1mutant constructs. The entire light chain genes were moved into pEF-BOS vector in their existing Kappa format from pCDNA vectors by HindIII restriction digest, filling the overhangs with T4 polymerase, followed by NotI digest. These blunt/NotI light chain fragments were then cloned into SrfI/NotI digested pEF-BOS vector.

V_H and V_L regions of antibodies were cloned from original hybridoma lines. RNA was prepared from antibody-producing cells, RT-PCR performed with primers designed as described above, i.e NruI/SalI primers for V_H, and NruI/BsiWI primers for V_L. The full length IgG1 and Kappa chains were assembled into cassette vectors.

The selected antibodies were further modified. Naturally occuring antibodies have either glutamine (Q) or glutamate (E) as the heavy and/or light chain NH2-terminus. Production of antibodies with Q as the NH2-terminus yields NH2-terminal heterogenity due to the cyclization of the glutamine residue to a glutamate. Therefore, the glutamine residue at the NH2-terminus of some of the antibodies was mutated to glutamate. Also two residues, Leucine234 and Leucine235 in the hinge region of the Fc portion, were mutated to prevent the effector functions of the antibody. Briefly, Leucine 234 and Leucine 235 were each replaced by an Alanine residue using standard molecular biological techniques(Lund, J. et al., *J. Immunology* (1991) 147: 2657-2662; Winter, *et al.* US PAT NOS 5,648,260; 5624821;5,624,821) . These Fc-mutated antibodies were termed (mg1). These mutants are further characterized in Examples 2.2.J 6, below.

Example 2.2.F: Characterization of Selected Neutralizing anti-IL-18 antibodies

Several recombinant anti-human IL-18 antibodies with distinct germ line sequences were produced in mammalian cells, purified and functionally characterized in various assays (see Table 6).

Table 6: In vitro antigen binding, cell assay and backbone sequence characteristics of some anti-IL-18 antibodies.

Antibody	Technology[a]	Biacore[b] (K_D, nM)	RBA (IC_{50}, nM)	KG-1[b] (IC_{50}, nM)	WBA (IC_{50}, nM)	Gene Families[c] Sequence Diversity	
2.5(E)mg1	Hybridoma	0.31	2.38	0.20	3	VL-L2	VH5-51
2.5(E)wtg1	Hybridoma	0.40	2.38	0.30	3	VL-L2	VH5-51
215(E)mg1[d]	Xenomax	0.23	1.17	0.17	3	VL-A27	VH4-31
444(Q)mg1[d]	Xenomax	1.61	2.49	0.13	1	VL-A27(7)	VH4-31(1)
581(E)mg1[d]	Xenomax	2.00	1.28	1.3	3	VL-A2	VH3-30
2.3.1(E)wtg1	Hybridoma	0.23	0.20	0.63	2	VL-02	VH4-59
2.13.1(E)wtg1	Hybridoma	0.20	0.20	0.12	2	VL-A27(8)	VH4-31(18)

NEM-cys protected rhuIL-18 was used.

Numbers in parenthesis indicate differences in amino acids from clone closet to germ line sequence

Example 2.2.G: Generation of CHO Cell Line Producing 2.5(E)mg1

Stable CHO cell lines expressing 2.5 (E)mg1 antibody were generated following the procedures outlined below.

Example 2.2.G 1: Construction of Expression Vector

The plasmid pA510 was constructed for high-level expression of antibodies in mammalian cell lines. This pUC19-derived plasmid contained the *E. coli* ColE1 origin of replication and the beta-lactamase gene for ampicillin resistance.

Briefly, cDNA corresponding to the VH and VL regions of the 2.5(E)mg1 antibody were cloned using standard molecular biological techniques, fused to mutated human gamma-1 and kappa constant region genes, respectively, such that DNA encoding a native, fully-human, IgG1/kappa antibody was produced. These DNAs were introduced into expression construct pA510. The resulting plasmid contained sequences (exclusive of pUC19) for the following genes or regulatory elements in the following order: 5′-CMV enhancer, adenovirus major late promoter, human immunoglobulin signal peptide, 2.5(E)mg1 heavy-chain immunoglobulin variable region, human gamma-1 immunoglobulin constant region, SV40 polyadenylation sequence, human gastrin transcription termination sequence, SV40 origin of replication (SV40 promoter/enhancer), murine dihydrofolate reductase sequence, thymidine kinase polyadenylation sequence from Herpes Simplex

virus, CMV enhancer, adenovirus major late promoter, human immunoglobulin signal peptide, 2.5(E)mg1 light-chain immunoglobulin variable region, human immunoglobulin kappa constant region, and SV40 polyadenylation sequence -3′. The coding regions were inserted downstream from strong viral promoters that drove the antibody gene transcription. The vector also encoded the expression of the mouse DHFR gene, which enabled selection of transformed cells by virtue their ability to grow in culture in the absence of nucleosides.

Example 2.2.G 2: **Transfection of Expression Vector Into Parental Cell Line**

The cell line, CHO DUX B11. (Urlaub, G. and Chasin L.A. *Proc Natl Acad Sci* USA 77: 4216-4220 (1980)), defective in the expression of the dihydrofolate reductase (DHFR) gene, was used for transfection of the expression vector described in Example 2.2.G 1. CHO DUX B11 cells were transfected with the vector using calcium phosphate precipitation method well known in the art (Current Protocols in Molecular Biology; Ausubel, F.V., Brent, R., Moore, D.M., Kingston, R.E., Seidman, J.G., Smith, J.A., and K. Struhl eds; Wiley Interscience, N.Y., N.Y. (1990)) with the following modifications. The plates were aspirated and 9 ml of F12 medium was added to each plate. The plates were incubated at 37^{0}C for two hours. One hundred and fifty micrograms of DNA were dissolved in 2.7ml water in a 50ml conical tube. Three hundred microliters of 2.5 M CaCl2 was added and this DNA mix was added one drop at a time to 3 ml of 2 x Hepes buffered saline (HeBS) in a 50 ml conical tube.

The resulting mixture was vortexed for 5 seconds and incubated at room temperature for 20 minutes. One ml was distributed evenly over each plate (still in F12) and the plates were incubated at 37^{0}C for four hours. After incubation, the plates were aspirated, and 2 ml of 10 % DMSO in F12 was added to each plate. The DMSO shock continued for one minute after which the DMSO was diluted by the addition of 5 ml of phosphate buffered saline (PBS) to each plate. The plates were aspirated and washed two more times in PBS. Ten ml of Gibco alpha MEM with nucleosides was added and the plates were incubated at 37^{0}C overnight. The next day, the medium was changed to Gibco alpha MEM without nucleosides with 5 % dialyzed fetal bovine serum (FBS), and six hours later the cells were seeded into 96-well plates as follows. The cells from the 10cm plates were harvested using trypsin digestion and resuspended in a total of 300 ml of Gibco alpha MEM without nucleosides with 5 % serum. Twenty, 96-well plates were seeded at 10 ml/plate, 100 l/well. One hundred ml of the same medium was added to the remaining 100 ml of cells and 20 additional 96-well plates were seeded as above. (This was a second dilution.) The medium was changed in the 96-

well plates one week later and again a week after that. The alpha MEM medium without nucleosides was used to select cells expressing DHFR and therefore the expression vector.

Example 2.2.G 3: Selection of 2.5(E)mg1 producing cells

Culture supernatants from transfected CHO cells were tested for the presence of secreted antibody 2.5(E)mg1 using an ELISA specific for human IgG. Once a set of CHO transfectants had been screened for expression of human antibody, an additional selection was used to isolate those cells that had amplified the number of copies of the expression vector integrated in the CHO genome. The drug methotrexate (MTX) was used for the selection of amplified lines. Cultures grown in the presence of MTX were tested for their ability to produce immunoglobulin. The MTX-resistant lines that expressed more antibody than their MTX-sensitive predecessors were taken through another cycle of selection in higher concentration MTX, and tested for immunoglobulin production. 2.5(E)mg1 expressing CHO cells were cultured in a 1 or 15 liter bioreactor and the yield of antibody was determined to be ~1.0g/L in a two-week run.

Example 2.2.H: Physicochemical Characterization of CHO Cell-Derived 2.5(E)mg1

Preliminary physical and chemical characterization of CHO derived 2.5(E)mg1 was performed. The experimentally determined molecular weight of 2.5(E)mg1 was approximately 149 kDa, in good agreement with the theoretical molecular weight. Using Peptide mapping techniques (K Biemann Annu. Rev. Biochem. 1992 61 977-1010; D A. Lewis Accelerated Articles, Anal. Chem. 1994, 66, 585-595) it was confirmed that 2.5(E)mg1 had the correct N-termini for both light and heavy chains. There was very little heavy chain C terminal variability, as 99% of the 2.5(E)mg1 molecules lacked lysine at the heavy chain carboxy termini. Each 2.5(E)mg1 heavy chain contained a single N-linked glycosylation site with oligomannose and complex, fucosylated binatennary structures with 0, 1 or 2 terminal galactose residues.

Example 2.2.I: Solubility and Stability of CHO Cell-Derived 2.5(E)mg1

Purified 2.5(E)mg1 was soluble to at least 62 mg/mL in pH 5, 6 and 7 buffers for a minimum of 4 weeks. Accelerated stability studies with 2.5(E)mg1 at 37°C in these buffers were performed to identify stability-indicating assays and the optimal long-term storage pH. Samples were taken at weekly intervals for analysis by size exclusion HPLC and SDS-PAGE to test for aggregation and fragmentation, LC-MS/MS peptide mapping for S-S bond detection, antigen-ELISA and/or cell based bioassay for activity measurement, and cation exchange HPLC and iso-Asp quantification for charge

heterogeneity measurement. Preliminary analysis of the samples by SEC (size exclusion chromatography), SDS-PAGE and cation-exchange chromatography showed that all three assays indicated stability and therefore 2.5(E)mg1 is more stable at ~pH6.

Example 2.2.J: Characterization of CHO-cell-derived anti-IL-18 HuMAb, 2.5(E)mg1

Example 2.2.J 1: IL-18 Species Specificity

The ability of 2.5(E)mg1 to bind and/or neutralize IL-18 from human, cynomolgus monkey, mouse, rat and dog was evaluated. Using the BIACORE assay following manufacturers intructions (see Example 2.1.B), it was shown that 2.5(E)mg1 bound mature human IL-18, but not mouse IL-18. In addition, immunoprecipitation data showed that 2.5(E)mg1 bound cynomolgus monkey IL-18 (IC50 for cyno IL-18= 9.1E X 10^{-11}), but not dog or rat IL-18. 2.5(E)mg1 functionally neutralized human and cynomolgus IL-18 bioactivity in a similar manner, but no inhibition of dog, rat or mouse IL-18 was seen.

Example 2.2.J 2: Human Cytokine Specificity

The specificity of 2.5(E)mg1 for IL-18 was evaluated using the BIACORE assay following manufacturers intructions (see Example 2.1.B). The 2.5(E)mg1 antibody was captured on the biosensor chip and its ability to bind a panel of known human cytokines in solution was determined. As shown in Table 7, 2.5(E)mg1 bound recombinant human mature IL-18 and pro-IL-18. In contrast, 2.5(E)mg1 did not bind to any of the other 23 human cytokines tested, including the IL-1 family members IL-1α and IL-1β.

Table 7 Biacore Analysis of Cytokine Binding by 2.5(E)mg1

Soluble rec. human cytokines, (1μM)	Captured 2.5(E)mg1 (25 mg/mL)
	2.5(E)mg1 Binding
IFNγ	-
IL-1α	-
IL-1β	-
Other cytokines[a]	-
IL-18[b]	+
Pro-IL-18	+

[a] Additional cytokines tested for binding included IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-21, TNF, LT, LTα1β2, and LTα2β1. 2.5(E)mg1 did not bind to any of these cytokines.

[b] Cysteine > Alanine mutant BV derived rec. human IL-18

Example 2.2.J 3: Affinity Measurements

Table 8 shows the *in vitro* IL-18 binding properties of 2.5(E)mg1 measured using the BIACORe assay according to manufacturer's instruction. The 2.5(E)mg1 antibody had a fast on-rate, slow off-rate with an overall affinity of 0.196nM. The kinetic rate parameters of two reference IL-18 antagonists (125-2H and IL-18 binding protein) are shown for comparison.

Table 8 IL-18 Binding Properties of 2.5(E)mg1 and Reference Reagents

Reagent	Biacore Parameters		
	On-rate ($x\ 10^3\ M^{-1}s^{-1}$)	Off-rate ($x\ 10^{-6}\ s^{-1}$)	K_D (nM)
2.5(E)mg1[a]	268	52.4	0.196
Murine anti-human IL-18 (125-2H)[b]	190	110	0.550
IL-18BP-Fc[c]	140	26	0.190

(4C/A)-HuIL-18 was tested in BIACORE.

[b] -125-2H, a neutralizing mouse anti-human IL-18 IgG1 mAb

[c] -IL-18BP-Fc, an Fc fusion of the natural IL-18 antagonist

Example 2.2.J 4: ***In Vitro* IL-18 Neutralization Potency**

The *in vitro* neutralization potency of 2.5(E)mg1 was determined in the KG-1 bioassay, the receptor binding assay (RBA) and the human WBA (see Example s 1.1.A-1.1.C). As shown in Table 8 the 2.5(E)mg1 antibody neutralized both recombinant (KG-1 and RBA) and natural IL-18 (WBA), (IC_{50} <0.5 nM in KG-1, <2 nM in RBA and <5 nM in WBA), and is consistent with its IL-18 binding affinity.

Table 8 Neutralization Potencies of 2.5(E)mg1 and Reference Reagents

Reagent	Neutralization Potency *in vitro* (IC50, nM)		
	KG-1[a]	RBA[b]	WBA[c]
2.5(E)mg1	0.2	2.4	3.0
Murine anti-human IL-18 mAb (125-2H)	0.2	>300[d]	3.0
IL-18BP-Fc	0.03	1.0	5.7
Anti-IL-18R mAb (M-840)	1.5	1.7	2.7

[b] KG-1 bioassay, mean values

[c] (4C/A)-huIL-18 was used in this assay

[d] Receptor Binding Assay

Human Whole blood assay with 4-6 individual donors. Mean values given.

125-2H neutralizes IL-18 bioactivity despite failure to inhibit receptor binding

Example 2.2.J 5: ***In Vivo* Neutralization Potency of 2.5(E)mg1**

To evaluate the ability of 2.5(E)mg1 to neutralize natural human IL-18-induced IFNγ in an inflammatory environment *in vivo*, the severe combined immunodeficient (SCID) mouse model was used, wherein human PBMCs were injected into the mouse and the cells were stimulated *in vivo* to produce human IL-18 (HuPBMC-SCID model). The results (Table 9) showed that 2.5(E)mg1 inhibited human IL-18-dependent human IFNγ production *in vivo* with a clear dose-response by either route of administration. The ED_{50} of 2.5(E)mg1 was approximately 1 μg or 0.1 μg / per mouse (=0.05 mg/kg or 0.005 mg/kg) by ip or iv administration, respectively.

Table 9A ***In vivo*** **efficacy of 2.5(E)mg1 administered i.p. in HuPBMC-SCID mouse model**

Group	huIFNg (pg/ml)	% Inhibition
2.5(E)mg1 0.025 μg/mouse	70 ± 17	61
2.5(E)mg1 0.25 μg/mouse	112 ± 29	36
2.5(E)mg1 2.5 μg/mouse	36 ± 10	80
2.5(E)mg1 25 μg/mouse	10 ± 8	94
2.5(E)mg1 250 μg/mouse	3 ± 2	98
No Treatment	193 ± 59	
HuIgG Control 250 μg/mouse	177 ± 33	

Table 9B ***In vivo*** **efficacy of 2.5(E)mg1 administered i.v. in HuPBMC-SCID mouse model**

Group	huIFNg (pg/ml)	% Inhibition
2.5(E)mg1 0.025 μg/mouse	156 ± 45	36
2.5(E)mg1 0.25 μg/mouse	27 ± 9	89
2.5(E)mg1 2.5 μg/mouse	36 ± 8	85
2.5(E)mg1 25 μg/mouse	11 ± 6	96
2.5(E)mg1 250 μg/mouse	4 ± 2	98
No Treatment	279 ± 26	
HuIgG Control 250 μg/mouse	245 ± 22	

Example 2.2.J 6: Effector Functions

The Fc portion of an antibody mediates several important effector functions e.g. cytokine induction, antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis, complement dependent cytotoxicity (CDC), and half-life/clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies

The L234A and L235A mutations in 2.5(E)mg1 did not influence the overall affinity or the neutralization potency of 2.5(E)mg1 HuMAb as compared with 2.5(E)wtg1 (Table 10). However, as expected, these mutations did abolish binding to FcγR and C1q.

Table 10: Mutations of residues L234 and L235 to Alanine does not affect affinity or neutralization potency of 2.5(E)mg1

	Kinetic Rate Parameters			KG-1 Bioassay
Ab	**On-rate (10^3 $M^{-1}s^{-1}$)**	**Off-rate (x 10-6 s^{-1})**	**K_D (nM)**	**IC_{50} (nM)**
2.5(E)wtg1	281	47.8	0.170	0.4
2.5(E)mg1	268	52.4	0.196	0.2

Example 2.2.J 6.1: <u>FcγR I binding</u>

The human FcγR I (CD64) has a relatively high affinity for IgG1 immune complexes (K_D 1E-8~1E-9 M). It is expressed on monocytes and macrophages and a number of myeloid cell lines including U937. The binding of 2.5(E)wtg1 and 2.5(E)mg1 to U937 cells was determined by fluorescence-activated cell sorting (FACS) (CURRENT PROTOCOLS IN IMMUNOLOGY. Vol (1) 5.3.1, Edited by J. E. Coligan et. al., Published by John Wiley & Sons, Inc., 2002). The data obtained (see Table 11) demonstrated that 2.5(E)wtg1 binds to U937 cells, but as expected, 2.5(E)mg1 did not. To confirm that this binding was mediated through FcγR I, a mouse anti-hFcγR I blocking antibody (10.1) was used for competition experiments. The result showed that antibody 10.1 blocked binding of 2.5(E)wtg1 to U937 cells in a dose-dependent manner at the concentrations tested below, and thus, 2.5(E)wtg1 binds FcγR I on U937 cells.

Table 11. Demonstration of the failure of 2.5(E)mg1, in contrast to 2.5(E)wtg1, to bind FcγR I on U937 cells (data shown as MFI+/-SD)

Antibody Conc (μM)	1.00E-09	1.00E-08	1.00E-07	1.00E-06	1.00E-05	1.00E-04	1.00E-03	1.00E-02	1.00E-01
2.5(E)wtg1	0.50+0.00	0.50+0.00	0.63+0.12	0.57+0.05	0.60+0.00	1.10+0.00	5.80+0.05	29.60+0.05	38.76+5.19
2.5(E)mg1	0.67+0.09	0.67+0.09	0.60+0.00	0.80+0.14	0.63+0.05	0.67+0.05	0.53+0.05	0.60+0.05	0.63+0.08

Example 2.2.J 6.2:FcγR II binding

The human FcγR II (CD32) has a relatively low binding affinity for IgG1 immune complexes (K_D 1E-5~1E-7M). Under physiological conditions, it requires the formation of multivalent immune complexes for activation. Using fluorescein isothiocyanate (FITC) labeled antibodies specific for FcγR I, II or III and detection by flow cytometry, we validated the expression of FcγR II on K562 cells and used this cell line for the FcγR II binding assay. The binding of monomeric 2.5(E)wtg1 to K562 cells was very weak. Therefore, an anti-kappa chain antibody was used to pre-crosslink the IgG1 molecules to mimic multivalent immune complexes and tested their binding to FcγR II on K562 cells. After cross-linking, 2.5(E)wtg1 bound to K562 cells, but even after cross-linking 2.5(E)mg1 showed only minimal, if any, binding (Table 12). An anti-FcγR II antibody, clone IV3, blocked binding of 2.5(E)wtg1, and thus, the 2.5(E)wtg1 binding to K562 was FcγR II mediated.

Table 12. Binding of 2.5(E)wtg1 and 2.5(E)mg1 to FcγR II on K562 cells after crosslinking (data shown as MFI+/-SD)

Antibody Conc (μM)	1.00E-08	1.00E-07	1.00E-06	1.00E-05	1.00E-04	1.00E-03	1.00E-02	1.00E-01	1.00E+00
2.5(E)wtg1	0.37+0.05	0.37+0.05	0.40+0.00	0.43+0.05	0.80+0.08	3.43+0.21	19.7+0.70	93.33+4.90	134.37+12.93
2.5(E)mg1	0.30+0.00	0.40+0.00	0.40+0.00	0.37+0.05	0.37+0.05	0.40+0.00	0.50+0.00	1.60+0.08	5.37+0.38

Example 2.2.J 6.3:C1q binding

Complement activation and lysis of the cells via the classic pathway is activated through binding of C1q to the Fc portion of IgG molecule. The binding of C1q to 2.5(E)wtg1 and 2.5(E)mg1 was determined using standard ELISA techniques known in the art (Hezareh, M., et. al., (2001) *J. Virology*,75 (24):12161-12168). 2.5(E)wtg1 and 2.5(E)mg1 HuMAbs were coated onto plastic plates followed by incubation with human C1q. Bound C1q molecules were then detected by a mixture of goat-anti-human C1q and rabbit anti-goat IgG alkaline phosphate conjugate. The results showed that 2.5(E)wtg1 bound C1q, but the 2.5(E)mg1 did not (Table 13).

Table 13. Demonstration of the failure of 2.5(E)mg1, in contrast to 2.5(E)wtg1, to bind to C1q by ELISA (data shown as OD_{405}+/-SD)

C1q Conc (μg/ml)	0	20	40	60	80	100	120
2.5(E)wtg1	0.09+0.00	0.78+0.00	0.98+0.00	1.06+0.07	1.14+0.06	1.32+0.13	1.24+0.06
2.5(E)mg1	0.10+0.01	0.12+0.00	0.16+0.00	0.18+0.00	0.21+0.01	0.21+0.01	0.22+0.00

Example 2.2.J 6.4:Neonatal Fc Receptor (FcRn) Binding

Interaction of IgG with the neonatal Fc receptor (also called Bramble receptor) in endothelial cells has been proposed to be an IgG quality control system and the critical determinant for the long half-life of IgGs [Ghetie, V., et al (1997) Nat. Biotechnol. 15:637-640]. IgG molecules taken up by pinocytosis and binding successfully to FcRn in endocytic vacuoles are returned to circulation. IgG molecules that fail to bind to FcRn are degraded.

The critical residues of human IgG for FcRn binding have been mapped to the junction of the CH2-CH3 domains (Kim J.K., et al (1999) Eur. J. Immunol. 29:2819-2825). Importantly, these FcRn binding residues are conserved between human and mouse immunoglobulins and human immunoglobulins bind to mouse FcRn allowing structure activity relationship studies in mice.

To test the effect of the L234A and L235A mutations on FcRn binding, binding of the wild-type 2.5(E)wtg1 and the mutant 2.5(E)mg1 to FcRn *in vitro* was determined using a FcRn expressing CHO cell line. 2.5(E)wtg1 and 2.5(E)mg1 were incubated with the FcRn expressing CHO cells at pH 6.5, followed by incubation with FITC-conjugated anti-human IgG (2° Ab). The cells were washed and analyzed by FACS.

The 500 nM concentration of 2.5(E)mg1 and 2.5(E)wtg1 showed significant binding to the FcRn compared to the 0.5 nM concentration, which was similar to background with cells alone.

Example 2.2.K: Pharmacokinetics in Mice

Pharmacokinetics (PK) of 2.5(E)mg1 were assessed in a screening mouse study to determine if the Fc mutations (L234A, L235A) introduced to prevent binding of 2.5(E)mg1 to FcγR and C1q adversely affected the serum PK profile. The mouse FcRn bound mouse and human IgG equally well making the mouse a relevant species for structure activity relationship studies in mice (Ober, R.J., et al (2001) Int. Immunol. 13:1551-1559). In mice, the terminal half-life of 2.5(E)mg1 was estimated to

be 12 days. In similar studies, the half-lives of other human monoclonal antibodies were 10 - 14 days.

The pharmacokinetics of 2.5(E)mg1 were evaluated in female mice (Jackson Labs, C57BL/6n) following a single intravenous dose of 0.2 mg (equivalent to an average of 10 mg/kg). A total of 24 mice were dosed and 3 samples were drawn from each mouse. The sampling scheme extended through seven days. 2.5(E)mg1 exhibited a distribution phase followed by an elimination phase. The distribution and elimination half-life estimates were approximately 1.6 hours and 12 days, based on a two compartment open model (Table 14).

Table 14 Summary of key pharmacokinetic parameters of 2.5(E)mg1 derived from a single intravenous dose in mice

$t_{1/2\alpha}$ (hr)	$t_{1/2\beta}$ (days)	C_{max} (g/mL)	CL (mL/hr)	V_{ss} (mL)	V_1 (mL)	V_2 (mL)	MRT (days)	AUC (hr*µg/mL)
1.58	12.2	63.2	0.0162	6.82	3.15	3.67	17.5	12250

Disease models

Example 2.2.L: Effect of anti-IL-18 antibodies in disease models

Example 2.2.L.1: Inhibition of LPS-induced IFNg production by anti-muIL-18 mAbs

LPS-induced IFNγ production is dependent upon IL-18 expression (Ghayur, T., et al, 1997. Nature 386:619-623.). An LPS-induced IFNγ production assay was used to determine the efficacy of 93-10C to inhibit IL-18-dependent LPS-induced IFNγ production *in vivo*. Mice were given a single iv dose of 93-10C (50 µg). Thirty minutes later mice were challenged with LPS (20 mg/kg) and bled 4h later. Serum IFNγ titers were determined by ELISA. As shown in Table 15, 93-10C inhibited LPS-induced IFNγ production by ~70%.

Table 15 93-10C inhibits LPS-induced IFNg production in vivo

Group	muIFNg (pg/ml)	% Inhibition
Rat IgG 250 µg/mouse	7239 ± 365	N/A
MBT 93-10C 250 µg/mouse	2395 ± 711	67

Example 2.2.L.2: **Inhibition of carrageenan-induced paw edema**

IL-18 is involved in neutrophil recruitment to sites of inflammation. Carrageenan-induced foodpad edema is a monocyte and neutrophil-dependent inflammation model. Edema in this model can be significantly inhibited by neutralizing the biological activity of IL-18 (Leung, B.P., et al (2001) J. Immunol. 167:2879-2886). Mice were dosed (ip) with 1C5 (400 μg) (Hyashibara Laboratories, Japan) or 93-10C (100 μg) (Medical and Biological Laboratories (MBL) Co. Watertown MA.)or control antibodies and then injected with carrageenan (sc) in the hind footpads. Carrageenan-induced edema was measured daily from 24 h to 96 h. 1C5 and 93-10C significantly suppressed carrageenan-induced edema (~50% inhibition) from 24h to 96 h post challenge (Table 16). In addition to blocking neutrophil infiltration, 93-10C also blocks TNF expression at the site of inflammation in this model (Leung, B.P., et al (2001) J. Immunol. 167:2879-2886).).

Table 16 *In vivo* suppression of carrageenan-induced paw edema

Carrageenan	Change in Paw Swelling (mm)			
Time (hrs)	24	48	72	96
125-2H @ 400	0.357	0.557	0.543	0.414
1C5 @ 400 ug	0.214	0.300	0.286	0.200
Rat IgG @ 100 ug	0.300	0.500	0.550	0.450
93-10C @ 100 ug	0.157	0.271	0.243	0.157
P<0.05 vs. Control IgG				

Example 2.2.L.3: **Collagen-induced arthritis**

Rheumatoid arthritis (RA) is characterized by chronic inflammation of joints, and, loss of bone and articular cartilage. Although RA is thought to be an autoimmune disease, the autoantigen involved has not been identified and the precise etiology of disease is unknown. Collagen-induced arthritis (CIA) is a widely used model of RA and has histopathological features which are similar to the human disease (Bendele, A., et al (1999) Toxicol Pathol. 27:134-142 ; Trentham, D.E. et al (1977) J. Exp. Med. 146:857-868). In this model, genetically-susceptible mice or rats are immunized with type II collagen (CII) in complete Freund's adjuvant. The resulting polyarthritis is characterized by destruction of cartilage, bone resorption, synovitis, and periarticular inflammation (Bendele, A., et al (1999) Toxicol Pathol. 27:134-142). IL-18 KO mice on a DBA/1 background showed decreased incidence and severity of CIA when compared to wild-type mice (Wei, X.Q., et al (2000) J. Immunol. 166:517-521).

To address the role of endogenous IL-18 in the pathogenesis of CIA, mice were treated with a rabbit polyclonal IgG (BA77) that neutralizes mouse IL-18. When dosed for 14 days from the time of priming, BA77 delayed disease onset and resulted in a significant decrease in disease severity. BA77 also significantly inhibited production of IgG2a anti-collagen antibodies. These results are similar to those reported for IL-18 KO mice and confirm a role for IL-18 as an important proinflammatory cytokine in early CIA.

The data from IL-18 KO mice and anti-IL-18 IgG treated wild type mice indicate that IL-18 plays an important proinflammatory role during CII-induced primary T cell activation. To better understand the role of IL-18 during the onset of CIA, mice were immunized with CII and treatment with rat IgG or 93-10C initiated just prior to disease onset, which occurs around day 14. Treatment with 93-10C resulted in a significant delay in disease onset and severity when compared to control rat IgG (Table 17). These data show that IL-18 is a significant factor not only in T cell priming but also in promoting arthritogenic responses after activation of CII-specific T cells.

Table 17 Anti-IL-18 mAb 93-10C delayed the onset and decreased the severity of CIA

Treatment	Mean Arthritic Score							
Day	11	12	13	14	15	16	17	18
Rat IgG @200 μg	0.00	0.13	0.13	0.13	0.27	0.53	1.20	1.20
93-10C @ 200 μg	0.00	0.00	0.00	0.00	0.07	0.00	0.20	0.47
Dexamethasone-21-P @1mpk	0.00	0.00	0.27	0.27	0.13	0.13	0.13	0.13
Treatment Period								
P<0.05 vs. Rat IgG								

Treatment	Mean Arthritic Score							
Day	19	20	21	22	23	25	26	27
Rat IgG @200 μg	1.53	1.73	1.93	2.27	2.53	4.20	4.27	4.53
93-10C @ 200 μg	0.47	0.80	1.00	1.00	1.13	2.20	2.27	2.87
Dexamethasone-21-P @1mpk	0.07	0.07	0.07	0.00	0.00	0.00	0.07	0.20
Treatment Period								
P<0.05 vs. Rat IgG								

Example 2.2.L.4: Septic Arthritis

IL-18 is an important factor in the pathogenesis of a mouse model of septic arthritis. This is generally not thought to be a model of RA, but shares some inflammatory components and pathology related to RA. In this model, disease is induced by injecting live group B streptococci (GBS) into knee joints. The severity of ensuing arthritis correlates with both systemic and local levels of IL-1β and IL-6, but not TNF (Tissi L., et al (1999) Infect. Immunol. 67:4545-50). Significant IL-18 levels in the joints were detected as early as 12 hours post injection with serotype IV (GBS) followed by peak IL-18 production after 5 days (~550 pg/ml in 1C5 treated vs ~30 pg/ml in IgG control). Elevated IL-18 levels were detected in the serum by day 5 post injection (~180 pg/ml in 1C5 treated vs ~20 pg/ml in IgG control).

When 1C5 was injected 1 hour prior to administration of GBS there was marked inhibition in the frequency of articular lesions from day 2 through day 10 (arthritic index: 1.0 in 1C5 treated vs 2.5 in IgG control). In addition, 1C5 treatment also resulted in significant reduction in cytokine levels in the joints including IL-6 and IL-1β. (Data not shown)

Example 2.2.L. 5: SLE

The most studied models of lupus involve strains of mice (MRL/lpr and NZB/NZW F1) that spontaneously develop lupus-like syndrome with severe glomerulonephritis, autoantibody production (anti-DNA, anti RNP etc.), splenomegaly, lymphadenopathy, and to some extent arthritis and vasculitis. Kidney involvement is observed usually at 3-5 months of age, progresses rapidly, and by 6-10 months is fatal. Both mouse strains have been extensively studied to gain an understanding of clinical disease.

The NZB/NZW F1 (B/W) mouse model (The Jackson Laboratory, Maine, USA) was selected as the most relevant model to evaluate the effects of exogenous IL-18 on lupus-like disease progression. The onset of disease progression in B/W mice is observed usually at 7-9 months of age and by 12-14 months is fatal as a result of renal failure. To investigate the role of IL-18 in lupus pathogenesis, B/W mice were treated daily with r-muIL-18 or vehicle control beginning at 7 months of age. Kidney function was assessed by determining the degree of proteinuria. Daily treatment of B/W mice with 50μg/kg of IL-18 led to accelerated onset of severe proteinuria as compared to the

PBS vehicle treated group. IL-18 treated B/W mice also exhibited accelerated deaths. These observations were consistent with those described above for the MRL/lpr mice and underscore a pro-inflammatory role for IL-18 in autoimmune disease.

To investigate the therapeutic effect of IL-18 blockade in a mouse model of SLE, an induction-maintenance treatment protocol in B/W mice that recapitulates clinical therapy for lupus nephritis was established. In this study, severely nephritic B/W mice received 5 weekly doses of cytoxan (induction phase) followed by chronic 1C5 or mouse IgG1 control (125-2H) treatment (maintenance phase).

Results demonstrate that in the ensuing 130 days of maintenance treatment, 1C5 significantly prolonged survival of BW mice as compared to control IgG1 125-2H. 125-2H is a mouse IgG1 mAb that does not recognize muIL-18 ($P < 0.05$,). In addition to the prolongation of survival, the onset of severe proteinuria was delayed, and there was a reduction of IgG2a and IgG1 anti-dsDNA in 1C5 treated BW mice. The reduction of anti-ds DNA by 1C5 treatment was transient and not statistically significant. Antibody against 1C5 (mouse anti-mouse antibody [MAMA]) was detected, and preceded the loss of efficacy after day 130, as evidenced by a precipitous drop in survival and the loss of effect in the reduction of anti-ds DNA titer and proteinuria. In conclusion, despite the presence of antibody responses to 1C5, IL-18 blockade by 1C5 prolonged survival, delayed onset of severe proteinuria, and reduced anti-ds DNA titers in B/W mice. These data demonstrate a role for IL-18 in promoting inflammatory responses resulting in a loss of kidney function and ultimately death.

Example 2.2.L. 6: <u>Multiple Sclerosis</u>

The contribution of IL-18 to the pathogenesis of experimental allergic encephalomyelitis (EAE; a murine model of MS) was investigated. Relapsing-remitting EAE is considered to be a relevant model for the human disease due to similar disease course, clinical signs, and CNS pathology. In these studies the disease was induced in IL-18 KO mice and WT C57/Bl6 mice. The IL-18 KO mice showed a slight delay in onset of disease symptoms compared to WT mice, and developed significantly less severe disease at later time points (Table 18). Treatment of WT mice with BA77 (anti-mouse IL-18 IgG (250 mg, 2X / wk), at day 0 through day 14 post immunization delayed the onset of disease symptoms and significantly limited disease severity at later time points

(Table 18). The protective effect of anti-IL-18 IgG could be observed at later time point even after cessation of treatment.

Table 18 Anti-IL-18 Ab treated mice and IL-18 knockout mice develop less severe EAE disease

		Mean Clinical Score Day 14 Post Immunization
PLP Induced EAE in SJL/J mice	IgG(BA77)	4
	anti-IL-18 ab	2.7
		Mean Clinical Score Day 18
MOG Induced EAE in IL-18 KO and WT mice	WT	3.6
	KO	2.1

Example 2.2.L.7: Liver Damage

Concanavalin A (Con A)-induced liver inflammation/damage is an animal model of T-cell-mediated liver disease. Activation of intra-hepatic T-cells by Con A leads to local production of inflammatory mediators (e.g. IFNγ and Fas ligand). Fas-Fas ligand interaction results in production of IL-18 that induces further IFNγ, Fas ligand and TNF production. Thus, a positive feedback loop is established that results in liver damage and excessive production of liver enzymes such as ALT and AST from dying cells. 1C5 or 93-10C mAbs were injected (ip) 1h prior to iv administration of 150 μg of Con A. Mice were bled 24h post Con A injection and serum titers of liver enzymes (ALT & AST) were determined. Both 1C5 and 93-10C blocked LPS-induced elevation of liver enzymes, although 93-10C was effective at lower doses (Table 19).

Table 19 *In vivo* inhibition of ConA-induced liver inflammation by 93-10C

Treatment	AST	stdv	ALT	stdv
PBS	57	16	30	2
ConA alone	1138	416	1294	481
ConA+93-10C (50ug)	183	70	153	88
ConA+93-10C (12.5ug)	635	427	443	256
ConA+rat IgG1(50ug)	3924	1062	3455	753

Example 2.2.L.8: Sepsis

IL-18 has emerged as an important mediator of endotoxic shock. IL-18 may be a critical mediator of endotoxin-induced lung, liver and multi-organ failure (Neeta, M.G., et al (2000) J. Immunol. 164:2644-2649). This effect of IL-18 may be dependent upon its ability to regulate production of cytotoxic mediators as well as its ability to activate innate immune responses and recruit neutrophils to the site of local inflammation. In addition, LPS challenge induces elevated serum levels of IFNγ, TNF and IL-1 and these cytokines may contribute towards LPS-induced lethality. IL-18 knockout mice challenged with LPS were deficient in LPS-induced IFNγ and produce significantly less TNF and IL-1 than WT mice (Takeda, K., et al. (1998) Immunity 8:383-390).

LPS induced lethality experiments were performed as follows. Animals were weighed on Day 0 and the appropriate dosage to be administered was determined. At T = -1 hour, animals were injected with anti-IL-18 antibodies or control antibodies in 500µl of 0.9% saline, Intra-peritoneal (IP). At T = 0, animals were injected with 20 mg/kg lipo poly saccharide (LPS) (E. coli serotype 0111:B4 Sigma Cat #L-4130 lot #71K4110) in 100µl of 0.9% saline, Intra-venous (IV). Four hours later blood was obtained from the animals via cardiac puncture. Serum muIFNγ titer was determined by muIFNγ ELISA (R&D Systems).

WT mice treated with anti-muIL-18 mAbs, 1C5 or 93-10C, were protected from LPS-induced lethality (Table 20) (125-2H, which has the same inactive isotype as 1C5 but does not bind muIL-18, served as the control). In addition, anti-IL-18 IgG treated mice were reported to have reduced lung and liver damage after LPS challenge and this correlated with reduced neutrophil accumulation(Neeta, M.G., et al (2000) J. Immunol. 164:2644-2649).

Table 20 1C5 and 93-10C mAbs prevent high dose LPS lethality

Lethality — Percent Survival

Time (hrs)	0	8	24	32	48	56	72	120	144
Saline	100	100	100	50	10	10	10	10	10
125-2H @ 400	100	100	80	70	10	10	10	10	10
1C5 @ 400 μg	100	100	100	90	80	80	80	80	80

Lethality — Percent Survival

Time (hrs)	0	8	24	32	48	56	72	120	144
Saline	100	100	90	40	10	10	10	10	10
Rat IgG @ 100 μg	100	100	100	100	70	40	40	40	40
93-10C @ 100 μg	100	100	100	100	100	100	100	100	100

Example 2.2.M: Crystallization of 2.5(E) Fab fragment

To demonstrate that the antibodies of the invention can be crystallized such that formulations and compositions comprising crystallized antibody can then be generated the following experiments were undertaken.

Example 2.2.M.1: Preparation and Purification of the 2.5(E) Antibody Fab Fragment

The 2.5(E) human IgG was expressed in CHO cells in SR-286 Media. The supernatant after lysis was filtered through a 0.5 micron filter and loaded onto a Protein A column pre-equilibrated in Protein A Buffer A (1XPBS). The IgG was then eluted with Protein A Buffer B (0.1 M Na Acetate pH 3.5, 150 mM NaCl). The pooled IgG was concentrated to 20 mg/ml, mixed with 50% papain gel slurry, and incubated at 37°C for 24 hours with vigorous shaking. The antibody/slurry mixture was then dialyzed against 50 mM Tris buffer pH 7.0 overnight at 4°C to remove cysteine from the buffer. A 25 mL Protein A Sepharose 4 Fast Flow affinity column (Amersham Pharmacia) was prepared by washing with with 100 mL of Buffer A (50 mM Tris pH 7.0). The dialyzed supernatant was applied to the affinity column (2 mL/min flow rate). 2.5(E)Fab fractions (monitored by UV absorbance at 280 nm) were collected in the flow-thru. Fractions containing a 2.5(E)Fab concentration greater than 0.3 mg/mL (determined by UV absorbance at 280 nm) were pooled and concentrated to ~20 mg/mL using an Ultrafree-15 Biomax 10 kDa molecular weight cut-off (MWCO) centrifugal filter device (Millipore) and frozen at –80 °C. This concentrated sample was used in crystallographic experiments described below. Sample purity was assessed with SDS-PAGE.

Example 2.2.M.2: Crystallization of the 2.5(E)Fab Fragment

Frozen 2.5(E)Fab stock solution (~20 mg/mL) was thawed on ice. The Fab (2 μL) was mixed with 2 μL of a reservoir solution consisting of 25–30% polyethyleneglycol (PEG) 400, 100 mM CAPS pH 10.5 and suspended over the reservoir on the underside of a siliconized glass cover slip at about 4 °C. Rod-like crystals appeared within one day. The rod-like crystals were determined to be 2.5(E) Fab fragment crystals (Data not shown).

Example 3: IL-18 responsive genes

Example 3.1: Materials and Methods

Throughout Example 4, the following materials and methods are used unless otherwise stated.

Example 3.1.A: Cell Treatment and RNA Preparation

Example 3.1.A.1: KG-1 cells

Approximately 3.0 x 10^7 KG1 cells (ATCC #CCL-246) were used for each experimental condition in four treatment groups. In the first, cells were treated with 50 ng/mL recombinant IL18 with or without a 30 min. preincubation with 10 mg/mL cycloheximide. After 30 min. or two hours cells were harvested for RNA. In the second, cells were treated with 0, 0.5, 2.0, 10 or 50 ng/mL recombinant IL18 with or without a 30 min. preincubation with 10 mg/mL cycloheximide. After two hours cells were harvested for RNA. In the third, cells were treated with 0 or 10 ng/mL TNF. Following an overnight incubation, cells were then treated with 0, 0.5, 2.0, 10 or 50 ng/mL recombinant IL18 with or without a 30 min. preincubation with 10 mg/mL cycloheximide. After two hours cells were harvested for RNA. In the final treatment group, cells were treated simultaneously with 0 or 10 ng/mL TNF and 0 or 2.0 ng/mL recombinant IL18 with or without a 30 min. preincubation with 10 mg/mL cycloheximide. After two hours cells were harvested for RNA.

Total RNA was prepared using TRIZOL Reagent (Life Technologies, Rockville, MD). An initial phase separation was performed according to the manufacturer's protocol and was followed by an additional extraction using a half volume of phenol: chloroform: isoamyl alcohol (25:24:1, Life Technologies, Rockville, MD). RNA precipitation and wash were performed according to the manufacturer's TRIZOL protocol instructions. Approximately 3 micrograms of RNA were electrophoresed on a 1.0% agarose/formaldehyde denaturing gel to assess quality.

For experiments requiring TNF preincubation, KG-1 cells were incubated for 12 hours with 2 ng/ml TNF prior to stimulation with 2, 10 or 40 ng/ml of IL18. RNA was prepared as described above.

Example 3.1.A.2: Human whole blood assays

2.5mL human whole blood was aliquoted into 15mL conical tubes and treated with IL18, IL12, IL18+IL12, IL18+IL12+anti-IL18 or IL18+IL12, IL18+IL12+control antibody . Final concentrations were as follows: IL12-500pg/mL, IL18(YK27-1)-50ng/mL, mIgG-5ug/mL, anti-IL18 1252H-5ug/mL, and anti-IL18 2.5-4ug/mL. Mixtures were incubated at 37°C for four hours with gentle intermittent inversion. After incubation, red blood cells were removed using ammonium chloride by adding 5mL 1Xlysis buffer (PharM Lyse Ammonium Chloride Lysing Reagent diluted 1:10 in Depc). After 5 minutes on ice the mixture was centrifuged at 1200 rpm for five minutes. This procedure was repeated once yielding a white pellet of blood leukocytes. RNA was isolated subsequently using the Trizol procedure described above. For micro array analysis, all sample volumes were increased by a factor of four.

Example 3.1.B: Preparation of Probe and Target Hybridization

Ten micrograms of total RNA and the SuperScript Choice System for cDNA Synthesis (Gibco BRL, Gaithersburg, MD) were used to synthesize double stranded cDNA. The synthesis was carried out according to Affymetrix (Santa Clara, CA) protocol, which requires T7-$(dT)_{24}$ oligomer primers (GENSET) in place of the oligo (dT) or random primers provided with the kit and incubations at 42°C during the temperature adjustment and first strand synthesis steps. The resulting cDNA was cleaned with Phase Lock Gel Light 2 ml tubes (Eppendorf AG, Hamburg, DE), and the pellet was suspended in 12 µL of DEPC-H_2O. 5 µL of the cDNA was used in conjunction with the BioArray High Yield RNA Transcript Labeling Kit (Enzo, Farmingdale, NY) to produce biotin-labeled cRNA targets by *in vitro* transcription (IVT) from T7 RNA polymerase promoters. Free nucleotides were removed from the IVT reaction with RNeasy Mini Columns (Qiagen, Hilden, DE). 15 µg of biotin-labeled cRNA was then fragmented according to Affymetrix protocol. The entire fragmented sample was combined with 5 µL control oligonucleotide B2 (Affymetrix), 15 µL 20X Eukaryotic Hybridization Control (Affymetrix), 3 µL sonicated salmon sperm DNA (10 mg/mL, Stratagene, La Jolla, CA), 3 µL acetylated BSA (50 mg/mL, Gibco BRL), 150 µL 2X MES hybridization buffer, and water to a final volume of 300 µL. Following Affymetrix protocol, Genechip HuGeneFL Arrays (Affymetrix) were pre-wet with 1X MES. The hybridization cocktails

were then heated and centrifuged, and 200 µL was loaded onto the chips. The chips were spun in a 45°C rotisserie oven for 16 hours.

Example 3.1.C: Washing, Staining, and Scanning Probe Arrays

The hybridization cocktail was removed from the chips and replaced with a non-stringent wash buffer. Chips were washed and stained using the EukGE-WS2 protocol on the GeneChip Fluidics Station 400, according to manufacturer's instruction (Affymetrix); a protocol that stained the chips with both Streptavidin Phycoerythrin (SAPE) stain solution and antibody solution. All necessary wash buffers and stains were prepared according to Affymetrix protocols. A GeneArray Scanner (Agilent, Palo Alto, CA) was used in conjunction with GeneChip software (Affymetrix) to scan the stained arrays.

Example 3.1.D: Data Analysis

Genechip data was transferred from Affymetrix MAS4 to Microsoft Excel then uploaded into Spotfire Decisionsite 7.0.

Example 3.2: Gene expression regulated by IL-18

Example 3.2.1: IL18 alone directly regulates a cohort of genes in KG1 cells.

To determine transcripts regulated directly by IL18, cytokine titration experiments were performed using KG1 cells in the presence and absence of the protein synthesis inhibitor cycloheximide. Shown in Table 1 is a list of 62 transcripts represented by 67 different probe sets (due to redundancies on the chip) found to have be regulated two fold or more with a *p* value of less than 0.05 (using Student's t test) under at least one condition in the presence and absence of cycloheximide. These genes comprised a variety of functional categories including transcription factors, kinases, and secreted proteins. Because these genes are regulated without de novo protein synthesis, these genes respond directly to IL18 induced signaling. Twelve genes encode secreted proteins, and thirteen encode surface molecules (making these feasible antibody targets). The remaining genes encode nuclear and cytoplasmic proteins (see Table 21).

Table 21. Genes induced by IL18.

Genbank ID	Location /function	Gene Name	Unigene Comment	0.5 ng/ml	2 ng/ml	10 ng/ml	50 ng/ml
L29217	kinase	CLK3	CDC-like kinase 3	9.1	7.4	8.1	15.0
D14497	kinase	MAP3K8	mitogen-activated protein kinase kinase kinase 8	6.6	2.9	5.8	3.9
L19871	neither	ATF3	activating transcription factor 3	1.0	1.1	3.3	2.6
U15460	neither	BATF	basic leucine zipper transcription factor, ATF-like	1.5	1.7	2.4	2.8
U45878	neither	BIRC3	baculoviral IAP repeat-containing 3	7.0	6.2	10.2	10.0
U37546	neither	BIRC3	baculoviral IAP repeat-containing 3	29.4	26.9	76.6	63.6
U72649	neither	BTG2	BTG family, member 2	3.1	4.7	6.6	5.9
L07765	neither	CES1	carboxylesterase 1	1.0	1.3	2.1	2.1
M27691	neither	CREB1	cAMP responsive element binding protein 1	0.9	2.4	4.9	3.1
HG3548-HT3749	neither	CUTL1	cut (CCAAT displacement protein)	2.5	2.1	1.3	0.7
X59131	neither	D13S106E	highly charged protein	2.1	0.5	1.5	2.3
U53445	neither	DOC1	downregulated in ovarian cancer 1	2.0	3.3	3.0	3.8
X68277	neither	DUSP1	dual specificity phosphatase 1	2.5	3.1	4.1	3.3
U48807	neither	DUSP4	dual specificity phosphatase 4	2.0	2.3	2.9	2.0
X52541	neither	EGR1	early growth response 1	15.5	12.7	32.4	20.3
X63741	neither	EGR3	early growth response 3	5.9	7.3	15.1	9.0
L07077	neither	EHHADH	enoyl-Coenzyme A	3.4	2.3	1.8	2.5
M62831	neither	ETR101	immediate early protein	3.4	5.8	6.3	6.8
L19314	neither	HRY	hairy (Drosophila)-homolog	2.3	2.5	2.3	2.0
S81914	neither	IER3	immediate early response 3	17.0	18.6	32.9	29.6
X51345	neither	JUNB	jun B proto-oncogene	7.2	6.1	10.7	9.6
U20734	neither	JUNB	jun B proto-oncogene	10.2	21.8	25.0	25.4
U49957	neither	LPP	LIM domain-containing	2.2	1.1	2.0	1.9
M58603	neither	NFKB1	nuclear factor kappa B (p105)	1.6	2.0	2.9	2.3
S76638	neither	NFKB2	nuclear factor kappa B	1.7	2.2	3.5	4.3
M69043	neither	NFKBIA	nuclear factor kappa B	9.6	10.4	15.5	15.8
U91616	neither	NFKBIE	nuclear factor kappa B	11.6	14.8	20.7	21.0
L13740	neither	NR4A1	nuclear receptor subfamily 4, group A, member 1	2.0	2.7	2.4	2.5
HG4115-HT4385	neither	OR1E3P	olfactory receptor	4.5	12.0	4.2	4.1
L20971	neither	PDE4B	phosphodiesterase 4B, cAMP-specific	2.4	2.8	4.2	3.5
U64675	neither	RANBP2L1	RAN binding protein 2-like 1	1.1	1.8	2.2	2.2
S57153	neither	RBBP1	retinoblastoma-binding protein 1	2.5	3.4	5.0	4.1
X75042	neither	REL	v-rel	1.6	2.5	3.9	3.7
M83221	neither	RELB	v-rel	2.3	2.8	2.8	2.6
S59049	neither	RGS1	regulator of G-protein signalling 1	10.9	12.7	22.4	17.8
U70426	neither	RGS16	regulator of G-protein signalling 16	3.9	4.7	7.5	6.7
U22377	neither	RLF	rearranged L-myc fusion sequence	2.5	2.0	2.5	2.6
M95787	neither	TAGLN	transgelin	6.6	4.7	1.0	1.6
L47345	neither	TCEB3	transcription elongation factor B (110kD, elongin A)	3.6	5.3	2.3	4.2
M59465	neither	TNFAIP3	tumor necrosis factor, alpha-induced protein 3	9.9	12.4	25.4	20.6
U19261	neither	TRAF1	TNF receptor-associated factor 1	2.8	2.8	4.9	4.1
U78798	neither	TRAF6	TNF receptor-associated factor 6	1.2	2.0	2.1	2.2
M37435	secreted	CSF1	colony stimulating factor 1 (macrophage)	2.9	2.9	2.1	2.6
M57731	secreted	GRO2	GRO2 oncogene	15.2	20.9	26.3	27.0
X53800	secreted	GRO3	GRO3 oncogene	4.1	5.5	14.8	9.9
X04500	secreted	IL1B	interleukin 1, beta	2.2	3.4	5.7	4.7
M28130	secreted	IL8	interleukin 8	6.2	10.0	13.4	14.5

Genbank ID	Location /function	Gene Name	Unigene Comment	0.5 ng/ml	2 ng/ml	10 ng/ml	50 ng/ml
Y00787	secreted	IL8	interleukin 8	5.8	7.4	8.3	8.5
U89922	secreted	LTB	lymphotoxin beta (TNF superfamily, member 3)	5.0	5.7	11.0	12.8
M31166	secreted	PTX3	pentaxin-related gene, rapidly induced by IL-1 beta	3.1	5.2	10.3	6.4
M23178	secreted	SCYA3	small inducible cytokine A3	1.8	2.0	5.0	3.8
M69203	secreted	SCYA4	small inducible cytokine A4	0.9	1.9	7.0	5.6
J04130	secreted	SCYA4	small inducible cytokine A4	1.0	2.6	5.9	4.5
M92357	secreted	TNFAIP2	tumor necrosis factor, alpha-induced protein 2	4.2	6.4	20.3	19.3
Z32765	surface	CD36	CD36 antigen (collagen type I/ TSP receptor)	1.6	2.0	1.4	1.2
Z11697	surface	CD83	CD83 antigen	4.7	8.2	19.6	16.7
M57730	surface	EFNA1	ephrin-A1	9.8	6.0	9.5	15.2
A28102	surface	GABRA3	gamma-aminobutyric acid (GABA) receptor	3.0	2.5	1.6	2.7
M24283	surface	ICAM1	intercellular adhesion molecule 1 (CD54)	7.5	11.5	14.5	13.9
M55024	surface	ICAM1	intercellular adhesion molecule 1 (CD54)	2.5	3.4	3.2	3.7
J03171	surface	IFNAR1	interferon (alpha, beta and omega) receptor 1	3.2	2.5	2.8	2.6
X01057	surface	IL2RA	interleukin 2 receptor, alpha	0.7	0.4	3.9	3.6
L10338	surface	SCN1B	sodium channel polypeptide	1.8	2.3	1.5	1.5
D79206	surface	SDC4	syndecan 4 (amphiglycan, ryudocan)	4.0	4.2	7.2	6.1
HG961-HT961	surface	SOS1	son of sevenless (Drosophila) homolog 1	6.3	6.2	9.1	9.9
X83490	surface	TNFRSF6	tumor necrosis factor receptor member 6	1.1	1.3	3.8	3.3
U19523	neither	GCH1	GTP cyclohydrolase 1		2.1		
U37518	surface	TNFSF10	tumor necrosis factor member 10	1.4	1.4	2.3	1.6

Example 3.2.2: **Cytokine exposure history effects KG-1 cell response to IL-18.**

Since cytokines typically appear sequentially during an immune response, the effect of preincubating KG-1 cells with TNF prior to treating with IL18 were tested. This experiment also tested the hypothesis that the cytokine exposure history of cells may effect their response to subsequent cytokine exposure. Cells were treated with 2ng TNF 12 hours prior to adding IL18 and harvested four hours later.

IL18 regulated the expression of approximately 125 genes under these conditions (Table 2). The filtering criteria used to obtain this set of genes was less than 50% change due to TNF and a two fold or greater change due to IL18 at the 10 ng/mL and 40 ng/mL. These genes comprised a variety of functional categories including transcription factors, kinases, and secreted proteins (Table 22). In contrast to other conditions tested here, we find interferon gamma mRNA and protein to be induced by IL18 following exposure to TNF.

Table 22. Genes regulated by IL18 following TNF treatment.

Genbank ID	Gene Name	Unigene Comment	Fold 10 ng	Fold 40 ng
J00219	IFNG	interferon, gamma	26.3	31.8
U17034	PLA2R1	phospholipase A2 receptor 1, 180kD	29.6	28.7
M57710	LGALS3	lectin, galactoside-binding, soluble, 3 (galectin 3)	27.5	25.4
X97748	PTX3	pentaxin-related gene, IL-1 induced	15.2	13.6
M27288	OSM	oncostatin M	23.1	12.0
X57809		lambda light chain variable region	10.9	10.0
Y00081	IL6	interleukin 6 (interferon, beta 2)	9.2	9.4
D16583	HDC	histidine decarboxylase	8.0	9.4
X07730	KLK3	kallikrein 3, (prostate specific antigen)	5.6	8.8
HG3111-HT3287		Homo sapiens clone HH409 unknown	9.5	7.5
M57732	TCF1	hepatic nuclear factor (HNF1)	2.0	7.2
U77735	PIM2	pim-2 oncogene	7.1	7.1
U96094	SLN	sarcolipin	12.2	6.1
D50640	PDE3B	phosphodiesterase 3B, cGMP-inhibited	4.0	5.4
X14008	LYZ	lysozyme (renal amyloidosis)	3.0	5.4
M91036	HBG2	hemoglobin, gamma G	3.4	5.4
X72755	MIG	monokine induced by gamma interferon	5.2	5.2
AC000099	GRM8	glutamate receptor, metabotropic 8	2.3	4.3
D11428	PMP22	peripheral myelin protein 22	5.0	4.0
M83667	CEBPD	CCAAT/enhancer binding protein (C/EBP), delta	4.3	4.0
L19267	DMWD	dystrophia myotonica, WD repeat motif	3.0	3.8
M81181	ATP1B2	ATPase, Na+/K+ transporting	3.5	3.8
U79249		Human clone 23839 sequence	3.1	3.7
U49973	FLJ10803	hypothetical protein FLJ10803	3.2	3.6
HG870-HT870	GOLGA3	golgi autoantigen, golgin subfamily a, 3	3.5	3.6
X13589	CYP19	cytochrome P450, subfamily XIX	3.0	3.5
AB000464		clone:RES4-24A	2.9	3.5
M96956	TDGF1	teratocarcinoma-derived growth factor 1	2.6	3.5
U31628	IL15RA	interleukin 15 receptor, alpha	6.4	3.3
D38128	PTGIR	prostaglandin I2 (prostacyclin) receptor (IP)	8.8	3.3
J03507	C7	complement component 7	2.3	3.1
M32011	NCF2	neutrophil cytosolic factor 2	3.5	3.0
X63131	PML	promyelocytic leukemia	4.7	3.0
D82326	SLC3A1	solute carrier family 3	4.0	3.0
L10343	PI3	protease inhibitor 3, skin-derived (SKALP)	2.1	3.0
U89995	FOXE1	forkhead box E1 (thyroid transcription factor 2)	2.6	2.9
M62800	SSA1	(52kD, ribonucleoprotein autoantigen SS-A/Ro)	3.1	2.9
AB000584	PLAB	prostate differentiation factor	2.4	2.8
U37519	ALDH8	aldehyde dehydrogenase 8	2.2	2.7
D21267	SNAP25	synaptosomal-associated protein, 25kD	2.2	2.7
M25667	GAP43	growth associated protein 43	2.5	2.7
L34357	GATA4	GATA-binding protein 4	2.3	2.7
U43944	ME1	malic enzyme 1, NADP(+)-dependent, cytosolic	3.0	2.7

Genbank ID	Gene Name	Unigene Comment	Fold 10 ng	Fold 40 ng
M16937	HOXB7	homeo box B7	2.9	2.6
U27326	FUT3	fucosyltransferase 3	2.6	2.6
Z23115	BCL2L1	BCL2-like 1	2.2	2.6
HG1877-HT1917	MBP	myelin basic protein	2.4	2.6
D10995	HTR1B	5-hydroxytryptamine (serotonin) receptor 1B	2.5	2.6
M91463	SLC2A4	solute carrier family 2 glucose transporter	3.1	2.5
U19878	TMEFF1	transmembrane with EGF and follistatin like	2.9	2.4
U66468	CGR11	cell growth regulatory with EF-hand domain	2.2	2.4
U44848	NRF1	nuclear respiratory factor 1	3.5	2.4
U73328	DLX4	distal-less homeobox 4	3.2	2.4
HG4593-HT4998		voltage-gated sodium channel (SCN1A)	2.3	2.4
X78710	MTF1	metal-regulatory transcription factor 1	2.7	2.4
X59727	MAPK4	mitogen-activated protein kinase 4	2.3	2.4
J03600	ALOX5	arachidonate 5-lipoxygenase	2.2	2.3
U87269	E4F1	E4F transcription factor 1	3.4	2.3
Y10375	PTPNS1	tyrosine phosphatase, non-receptor substrate 1	4.5	2.2
D49958	GPM6A	glycoprotein M6A	3.3	2.2
U60062	FEZ1	fasciculation & elongation protein zeta 1 (zygin I)	3.3	2.2
X14830	CHRNB1	cholinergic receptor, nicotinic, beta polypeptide 1	2.4	2.1
J04076	EGR2	early growth response 2 (Krox-20 homolog)	3.0	2.1
HG2981-HT3127	CD44	CD44 antigen	2.2	2.1
U49187	C6ORF32	chromosome 6 open reading frame 32	3.8	2.1
X77744		Homo sapiens for FLJ00032 protein, partial	2.3	2.1
X68285	GK	glycerol kinase	2.4	2.0
HG3925-HT4195	SFTPA2	surfactant, pulmonary-associated protein A2	3.9	2.0
M26062	IL2RB	interleukin 2 receptor, beta	0.2	0.5
X06182	KIT	v-kit oncogene homolog	0.4	0.5
U79251	OPCML	opioid-binding protein/cell adhesion molecule-like	0.5	0.5
J03764	SERPINE1	nexin, plasminogen activator inhibitor type 1	0.5	0.5
X92814	HREV107	similar to rat HREV107	0.3	0.5
L01087	PRKCQ	protein kinase C, theta	0.2	0.5
D43772	GRB7	growth factor receptor-bound protein 7	0.2	0.5
X15880	COL6A1	collagen, type VI, alpha 1	0.5	0.5
HG3115-HT3291	MBP	myelin basic protein	0.4	0.5
X83301	SMA3	SMA3	0.5	0.5
D87469	CELSR2	cadherin, EGF LAG seven-pass G-type receptor 2	0.4	0.5
M11313	A2M	alpha-2-macroglobulin	0.4	0.4
X64877	HFL3	H factor (complement)-like 3	0.4	0.4
Z18859	GNAT2	guanine nucleotide binding protein (G protein)	0.4	0.4
D89077	SLA	Src-like-adapter	0.4	0.4
L25444	TAF2E	TATA box binding protein (TBP)-associated factor	0.2	0.4
M26665	HTN3	histatin 3	0.4	0.4
S69790	WASF3	WAS protein family, member 3	0.4	0.4
U79248		Human clone 23826 sequence	0.4	0.4
L15309	ZNF141	zinc finger protein 141 (clone pHZ-44)	0.3	0.4
L41147	HTR6	5-hydroxytryptamine (serotonin) receptor 6	0.4	0.4
X58431	HOXB6	homeo box B6	0.4	0.4

Genbank ID	Gene Name	Unigene Comment	Fold 10 ng	Fold 40 ng
U50360	CAMK2G	CaM kinase II gamma	0.2	0.4
D88152	ACATN	acetyl-Coenzyme A transporter	0.4	0.4
U38480	RXRG	retinoid X receptor, gamma	0.3	0.4
X16866	CYP2D7AP	cytochrome P450, subfamily IID	0.4	0.4
X70991	NAB2	NGFI-A binding protein 2 (ERG1 bp 2)	0.2	0.4
M60830	EVI2B	ecotropic viral integration site 2B	0.4	0.4
M27492	IL1R1	interleukin 1 receptor, type I	0.4	0.4
Z35093	SURF1	surfeit 1	0.4	0.4
D86425	NID2	nidogen 2	0.3	0.3
U59914	MADH6	MAD) homolog 6	0.4	0.3
M18255	PRKCB1	protein kinase C, beta 1	0.4	0.3
AF000234	P2RX4	purinergic receptor P2X	0.3	0.3
S77763	NFE2	nuclear factor (erythroid-derived 2), 45kD	0.4	0.3
U78722	ZNF165	zinc finger protein 165	0.3	0.3
L05568	SLC6A4	solute carrier family 6 (serotonin),	0.3	0.3
L31529	SNTB1	syntrophin, dystrophin-associated protein A1,	0.3	0.3
U47054	ART3	ADP-ribosyltransferase 3	0.4	0.3
M13955	KRT7	keratin 7	0.4	0.3
D15049	PTPRH	protein tyrosine phosphatase, receptor type, H	0.4	0.3
U03486	GJA5	gap junction protein, alpha 5, 40kD (connexin 40)	0.5	0.3
X06256	ITGA5	integrin, alpha 5	0.4	0.3
U22314	REST	RE1-silencing transcription factor	0.3	0.3
U51096	CDX2	caudal type homeo box transcription factor 2	0.2	0.2
D31762	KIAA0057	TRAM-like protein	0.4	0.2
M23668	FDX1	ferredoxin 1	0.2	0.2
U53476	WNT7A	wingless-type MMTV integration site family	0.2	0.2
X57206	ITPKB	inositol 1,4,5-trisphosphate 3-kinase B	0.2	0.2
Z31695	INPP5A	inositol polyphosphate-5-phosphatase, 40kD	0.4	0.2
S66793	ARR3	arrestin 3, retinal (X-arrestin)	0.2	0.2
U59877	RAB31	RAB31, member RAS oncogene family	0.2	0.2
U53786	EVPL	envoplakin	0.2	0.2
S83362	LIFR	leukemia inhibitory factor receptor	0.3	0.2
D42038	KIAA0087	KIAA0087 gene product	0.3	0.2
HG4333-HT4603	ZNF79	zinc finger protein 79 (pT7)	0.1	0.1
L01406	GHRHR	growth hormone releasing hormone receptor	0.4	0.1

Example 3.2.3: **Human Leukocyte Response to IL18.**

The response of human leukocytes (isolated leukophoresis) to respond to IL18 alone or in combination with IL12 was tested. The ability of an anti-IL18 monoclonal antibody to inhibit the transcriptional response was also tested. Cells were treated as described Example 4.1. RNA was isolated and used to probe Affymetrix Genechips (Hugene, FL). The results are shown in Table 23, which lists 49 transcripts induced by IL18+IL12 and reversed by anti-IL18 antibody. Several genes were relevant to the immune system. Many of these genes were also induced by IL18 in KG-1 cells.

Table 23. Other potential IL18/IL12 markers selected transcripts upregulated four fold or more by IL18+IL12 and reversed by 1252H in a human leukocyte sample as determined using Affymetrix Genechips.

Gene Name	Unigene Comment	Unigene
KIAA0001	putative G protein coupled receptor for UDP-glucose	Hs.2465
LIMK2	LIM domain kinase 2	Hs.278027
KIAA0196	KIAA0196 gene product	Hs.8294
IFNG	interferon, gamma	Hs.856
POLR2C	polymerase (RNA) II polypeptide	Hs.79402
DAG1	dystroglycan 1	Hs.76111
TPSB1	tryptase beta 1	Hs.250700
CDR2	cerebellar degeneration-related protein (62kD)	Hs.75124
TCF12	helix-loop-helix transcription factors 4	Hs.21704
TACTILE	T cell activation, increased late expression	Hs.142023
PIP5K2A	phosphatidylinositol-4-phosphate 5-kinase	Hs.108966
SF3A3	splicing factor 3a, subunit 3, 60kD	Hs.77897
SEL1L	sel-1 (suppressor of lin-12, C.elegans)-like	Hs.181300
IL15	interleukin 15	Hs.168132
BAK1	BCL2-antagonist/killer 1	Hs.93213
SLAM	signaling lymphocytic activation molecule	Hs.32970
SCYB11	small inducible cytokine subfamily B (Cys-X-Cys), member 11	Hs.103982
LIMK1	LIM domain kinase 1	Hs.36566
CAT56	CAT56 protein	Hs.118354
POLRMT	polymerase (RNA) mitochondrial (DNA directed)	Hs.153880
SCYA4	small inducible cytokine A4/Mip-1b	Hs.75703
MIG	monokine induced by gamma interferon	Hs.77367
SSX3	synovial sarcoma, X breakpoint 3	Hs.178749
TNFRSF6	tumor necrosis factor receptor superfamily, member 6	Hs.82359
MAT1A	methionine adenosyltransferase I, alpha	Hs.323715
KIAA0133	KIAA0133 gene product	Hs.57730
FCGBP	Fc fragment of IgG binding protein	Hs.111732
ARHD	ras homolog gene family, member	Hs.15114
FGFR2	fibroblast growth factor receptor 2	Hs.278581
COL9A1	collagen, type IX, alpha 1	Hs.154850
HPX42B	haemopoietic progenitor homeobox	Hs.125231
TAL2	T-cell acute lymphocytic leukemia 2	Hs.247978
	ESTs	Hs.196244
REN	renin	Hs.3210
POU2F2	POU domain, class 2, transcription factor 2	Hs.1101
ALOX12	arachidonate 12-lipoxygenase	Hs.1200

Gene Name	Unigene Comment	Unigene
ACTN2	actinin, alpha 2	Hs.83672
KLK2	kallikrein 2, prostatic	Hs.181350
RCV1	recoverin	Hs.80539
E2F4	E2F transcription factor 4, p107/p130-binding	Hs.108371
SEMA3F	immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F	Hs.32981
BHMT	betaine-homocysteine methyltransferase	Hs.80756
EVPL	envoplakin	Hs.25482
BBC3	Bcl-2 binding component 3	Hs.87246
SLN	sarcolipin	Hs.15219
RDBP	RD RNA-binding protein	Hs.106061
MT1H	metallothionein 1H	Hs.2667
RAD54L	RAD54 (S.cerevisiae)-like	Hs.66718
MLL3	myeloid/lymphoid or mixed-lineage leukemia3	Hs.288971

Example 3.2.4: Human whole blood response to IL18.

The response of whole human blood to respond to IL18 alone or in combination with IL12 was tested. The ability of an anti-IL18 monoclonal antibody to inhibit the transcriptional response was also tested. Normal donor blood samples were treated as described in Example 4.1. RNA was isolated and used to probe Affymetrix Genechips (HugeneFL). The results are shown in Table 24 which lists 16 transcripts that were significantly regulated by IL18+IL12 and reversed by anti-IL18 antibody in whole blood samples isolated from two healthy donors. Several genes were relevant to the immune system. We went on to test the response of three of these genes in panel of 10 normal donors using quantitative PCR. The results of this human variability study are shown in Table 25, for interferon gamma; Table 26, CXCL9 and Table 27, CCL8. The results of the variability study indicated that regulation of these transcripts by IL18 in human blood is likely to be a common among humans.

Table 24. Other potential IL18/IL12 markers selected from transcripts up-regulated in whole blood isolated from two donors then treated with IL18+IL12.

Probe Set ID	Title	Unigene
202284_s_at	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	Hs.179665
202531_at	interferon regulatory factor 1	Hs.80645
204057_at	interferon consensus sequence binding protein 1	Hs.14453
205488_at	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	Hs.90708
206554_x_at	SET domain and mariner transposase fusion gene	Hs.265855

206817_x_at	trinucleotide repeat containing 4	Hs.26047
207509_s_at	leukocyte-associated Ig-like receptor 2	Hs.43803
209546_s_at	apolipoprotein L, 1	Hs.114309
214438_at	H2.0-like homeo box 1 (Drosophila)	Hs.74870
214450_at	cathepsin W (lymphopain)	Hs.87450
216950_s_at	FcRI b form (AA 1-344) [Homo sapiens], mRNA sequence	Hs.382006
217933_s_at	leucine aminopeptidase 3	Hs.182579
219386_s_at	B lymphocyte activator macrophage expressed	Hs.20450
219956_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 6	Hs.151678
219971_at	interleukin 21 receptor	Hs.210546
221223_x_at	cytokine inducible SH2-containing protein	Hs.8257

Table 25. Interferon γ performance in ten human blood samples. $p<0.05$ for inhibition by either antibody

IFN	Unstimulated	Stimulated	Anti-IL18 2.5	Anti-IL18 125-2H
donor 3n	0.001	0.187	0.014	0.026
donor 5n	0.003	0.012	0.006	0.006
donor 9n	0.001	1.250	0.037	0.000
donor10n	0.002	0.361	0.024	0.002
donor1n	0.002	0.339	0.022	0.070
donor2n	0.001	0.032	0.003	0.003
donor4n	0.001	0.082	0.011	0.027
donor6n	0.002	0.076	0.006	0.010
donor7n	0.002	0.049	0.009	0.012
donor8n	0.002	0.049	0.009	0.012

Table 26. MIG/CXCL9 performance in ten human blood samples. $p<0.05$ for inhibition by either antibody.

CXCL9	Unstimulated	Stimulated	Anti-IL18 2.5	Anti-IL18 125-2H
donor1	0.000	0.170	0.082	0.010
donor10	0.000	0.015	0.000	0.000
donor2	0.001	0.006	0.001	0.001
donor3	0.000	0.067	0.010	0.006
donor4	0.000	0.023	0.012	0.003
donor5	0.000	0.004	0.000	0.000
donor6	0.000	0.070	0.001	0.001
donor7	0.001	0.034	0.001	0.000
donor8	0.001	0.034	0.001	0.000
donor9	0.000	0.035	0.000	0.001

Table 27. MCP2/CCL8 performance in ten human blood samples. $p<0.05$ for inhibition by either antibody.

CCL8	Unstimulated	Stimulated	Anti-IL18 2.5	Anti-IL18 125-2H
donor1	0.036	8.941	4.054	1.051
donor10	0.004	0.987	0.009	0.025
donor2	0.036	1.225	0.105	0.057
donor3	0.012	3.923	0.648	0.663
donor4	0.021	2.227	0.994	0.630
donor5	0.001	0.005	0.001	0.001
donor6	0.000	0.023	0.002	0.001
donor7	0.001	0.009	0.001	0.001
donor8	0.001	0.009	0.001	0.001
donor9	0.001	2.438	0.003	0.059

The present invention incorporates by reference in their entirety techniques well known in the field of molecular biology. These techniques include, but are not limited to, techniques described in the following publications:

Ausubel, F.M. et al. eds., Short Protocols In Molecular Biology (4th Ed. 1999) John Wiley & Sons, NY. (ISBN 0-471-32938-X).

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Although a number of embodiments and features have been described above, it will be understood by those skilled in the art that modifications and variations of the described embodiments and features may be made without departing from the present disclosure or the invention as defined in the appended claims. Each of the publications mentioned herein is incorporated by reference.